THE
BOTANICAL GAZETTE

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Margaret MacLeod

The passing of Miss Margaret MacLeod has removed from the field of botanical science one who has contributed much to it. Beginning in 1913, she was continuously associated with the BOTANICAL GAZETTE as assistant to its successive editors. Her indefatigable labor and her high standards of accuracy and perfection gave the Journal its essential quality, particularly during recent years. Through her friendliness and character she merited and received the sincere admiration and regard of her associates. Many knew her only through correspondence. To her friends and co-workers the personal loss is very great.

Margaret MacLeod was born in Loughborough, Leicestershire, England. She came directly from there to Chicago in 1910. In America she is survived by a sister, Mrs. Cecil Moss, Stratford, Ontario, Canada. All her other relatives are in England.

E. J. KRAUS

CYTO-TAXONOMIC STUDIES IN ORYZOPSIS

B. LENNART JOHNSON

Introduction

The genus *Oryzopsis*, like other genera of the tribe Agrostideae, is difficult to circumscribe satisfactorily. It is characterized by discontinuities between small groups of closely related species. On some characters it approaches the neighboring genera *Nassella* and *Piptochaetium*, while on others its close affinity to *Stipa* has resulted in an almost arbitrary assignment of certain species to either genus. Three main aggregates of species within *Oryzopsis* have in the past been recognized as different genera or have been combined with *Nassella* and *Piptochaetium* to form a more comprehensive genus. The whole problem of where to draw the generic boundaries in this part of the subtribe Stipeae is still an open one. Whether *Oryzopsis* is a natural genus is a question which will bear much weight in the ultimate disposition of its component species, as well as of the smaller adjacent genera.

The history of *Oryzopsis* bears out the fact that considerable uncertainty exists as to its proper circumscription. The genus was founded by MICHAUX (12) in 1803 on the basis of a single species, *Oryzopsis asperifolia* Michx., whose range he cited as Hudson Bay to Quebec. Previous to that date four Old World species from the general region of the Mediterranean now included in *Oryzopsis* had been described and referred to *Agrostis* and *Milium*. In 1812 BEAUVOIS (3) established the genus *Piptatherum*, which eventually included these species of *Milium* and *Agrostis* as well as two

which were described later. The western American species now known as *Oryzopsis hymenoides* (Roem. and Schult.) Ricker was described as a *Stipa*, but in 1818 NUTTALL (14) founded the genus *Eriocoma* on the basis of this single species.

The three genera *Oryzopsis*, *Piptatherum*, and *Eriocoma* were subsequently reduced to the status of sections in the comprehensive genus *Urachne* Trin., which was established in 1820. The genus *Piptochaetium*, which had been founded by PRESL (15) in 1830, was next reduced to sectional rank and included in *Urachne*. To these four sections TRINIUS then added the section *Nassella*. The last two sections consist mostly of temperate South American species. Thus the genus *Urachne* included all the present species of *Oryzopsis* which had been described under various genera up to that time. In addition, it included the sections *Piptochaetium* and *Nassella*, which are now excluded from *Oryzopsis*.

BENTHAM (4) in 1882 recognized that *Urachne* was a synonym of *Oryzopsis* and adopted the latter name. He retained the sections *Piptatherum* and *Eriocoma* as defined by TRINIUS but included *Piptochaetium* and *Nassella* in his section *Euoryzopsis*. HACKEL (6) later excluded *Piptochaetium* and *Nassella* from the section *Euoryzopsis*, giving them generic rank. This left the genus *Oryzopsis* with three sections, *Euoryzopsis*, *Piptatherum*, and *Eriocoma*, an interpretation which has persisted up to the present time.

In contrast to this complicated derivation of the present genus *Oryzopsis* is that of the closely allied genus *Stipa*. Less than 10% of the species of *Stipa* treated by HITCHCOCK (9) have been referred to other genera at any time, while all the species of *Oryzopsis* considered in this study—except the last two to be described—have borne from one to four different generic names. Only four of them were originally described under *Oryzopsis*. In the opinion of HITCHCOCK (8), *Stipa*, *Oryzopsis*, *Nassella*, and *Piptochaetium* form a "well-marked group" in the subtribe Stipeae. The contrast in their treatment by systematists suggests that *Stipa* is a large, coherent swarm of species and that the other genera represent smaller peripheral swarms whose precise natural positions are not clear.

Judging in part from the routine transfers that have been made in the past between *Oryzopsis* and other genera, it is obvious that a continuation of the same practice will not alone clarify the generic entities in this general group. A critical re-examination of morphological characters is desirable in order to evaluate the degree of relationship within and between these aggregates of species.

Discontinuities in general are largely the result of isolation, and changes in the chromosomal complement frequently act as efficient isolating mechanisms. Thus, if the information gained by re-examining morphological characters is supplemented by a knowledge of the nature of the interrelationships as revealed by cytological studies, it should be possible to draw the generic boundaries with greater confidence. With this aim, the present cyto-taxonomic study of *Oryzopsis* is undertaken, giving special attention to its confusing points of contact with *Stipa*.

Material and methods

Data on morphological characters were obtained from herbarium sheets selected by the use of TIPPETT's (17) random sampling numbers so as to represent in general the geographic ranges of the various species studied. The information presented in tables 1–10 is in most cases based on measurements from two spikelets obtained from each specimen with the aid of TIPPETT's numbers. The illustrations of floral parts, except in the case of *Oryzopsis hymenoides*, were prepared from photographs made at a uniform magnification for all species.

Cytological materials were obtained from plants transplanted directly to the greenhouse from their natural habitat or from nursery plots. In a few cases plants were grown from seed. Root tips were uniformly fixed in Randolph's chromo-acetic-formalin. Camera-lucida drawings were made of root-tip metaphase figures at a magnification of 2420 ×. The number of drawings made varied from twenty-five in some of the species with low chromosome numbers to six in the species with the highest chromosome number. In most cases the number of satisfactory counts far exceeded the number of drawings made. The idiogram for each species shown in figures 24–32 is based on the average of three such drawings. For each drawing, the chromosomes were measured on a millimeter scale and estimated to the nearest tenth of a millimeter, paired on the basis of length and gross morphology, and arranged in order of decreasing length. These chromosome lengths were then averaged for the three drawings. Only the longer chromosome of each pair is illustrated.

Taxonomic summary of Oryzopsis

Characters of the lemma are in general relied upon to distinguish *Oryzopsis*

from *Stipa*. In *Oryzopsis* the lemma is relatively short, broad, and usually indurate, with a blunt callus and deciduous awn. In *Stipa* the lemma is long and slender, usually less indurate, with a sharp-pointed callus and a persistent awn. Also, the panicle in *Oryzopsis* is usually open, while in *Stipa* it is usually contracted. As is frequently the case, none of these characters is wholly reliable when considered independently. Even when considered together, they do not effect a clear distinction between the two genera, for certain species are either intermediate in the expression of a majority of these characters or else distinctly resemble *Oryzopsis* in some and *Stipa* in others. The tendency on the part of systematists has been to assign the doubtful cases to *Oryzopsis*.

Intergradation of these two genera occurs in North America in the sections *Euoryzopsis* and *Eriocoma*. The Old World species are included in the section *Piptatherum*, and they constitute a clearly defined group which cannot readily be confused with *Stipa*.

Sections of Oryzopsis

Section 1.—*Piptatherum* (Beauv.) Benth.

Lemma obovate or elliptic, mostly dorsiventrally compressed and greatly indurate (not indurate in *O. miliacea*), glabrous or short pubescent, pale to dark brown or black, without a distinct callus; awn straight or flexuous and deciduous; palea at maturity broadly exposed between edges of lemma; glumes acuminate or acute, exceeding the lemma, 3–9 nerved, first glume longer than or equal to second; panicle open, branches distant and spreading, spikelet-bearing toward the ends; style branches reflexed at anthesis.

Oryzopsis miliacea (L.) Benth. and Hook.
O. virescens (Scop.) Beck.
O. paradoxa (L.) Nutt.
O. coerulescens (Desf.) Hack.
O. holciformis (Bieb.) Hack.
O. racemosa (J. E. Smith) Ricker

Section 2.—*Eriocoma* (Nutt.) Benth.

Lemma broadly fusiform, indurate, villous dark brown at maturity, with distinct, oblique callus; awn straight and deciduous; palea at maturity exposed between edges of lemma; glumes acuminate, greatly exceeding the lemma, 3–7 nerved, panicle diffuse with long capillary pedicels or contracted with erect branches and pedicels.

Oryzopsis hymenoides (Roem. and Schult.) Ricker

Section 3.—*Euoryzopsis* Benth.

Lemma obovate or fusiform, not greatly indurate, pubescent and pale or dark brown at maturity (glabrous in *O. hendersoni* and mostly glabrous in *O. micrantha*), with short callus; awn straight to geniculate, deciduous or fairly persistent; palea at maturity narrowly exposed between edges of lemma (or completely inclosed in convolute lemma in *O. asperifolia* and *O. hendersoni*); glumes obtuse or acute, equal to the lemma (acuminate and exceeding the lemma in *O. micrantha*), mostly 1–3 nerved (5–9 nerved in *O. asperifolia* and *O. hendersoni*), second glume longer than or equal to first; panicle narrow or open; style branches erect at anthesis.

Oryzopsis micrantha (Trin. and Rupr.) Thurb.
O. pungens (Torr.) Hitchc.
O. canadensis (Poir.) Torr.
O. exigua Thurb.
O. kingii (Boland.) Beal
O. asperifolia Michx.
O. hendersoni Vasey

KEY TO NORTH AMERICAN SPECIES
OF ORYZOPSIS

1. Glumes longer than lemma, at least 3-nerved, equal or the first exceeding the second
  2. Lemma glabrous or short pubescent with hairs less than 0.5 mm. long, somewhat dorsiventrally compressed, with indistinct callus........Section *Piptatherum*
    3. Lemma less than 3 mm. long, glabrous, pale at maturity (introduced)
      O. miliacea
    3. Lemma more than 6 mm. long, short pubescent, black at maturity
      O. racemosa
  2. Lemma pubescent with hairs more than 1 mm. long, fusiform, with distinct callus
    4. Lemma less than 4 mm. long; awn straight, less than 9 mm. long
      Section *Eriocoma*
      5. Panicle mostly diffuse (rarely contracted); hairs of lemma more than 2 mm. long........*O. hymenoides*
      5. Panicle contracted with erect branches; hairs of lemma less than 2 mm. long
        O. hymenoides var. *contracta*
    4. Lemma more than 4 mm. long; awn once geniculate, more than 10 mm. long (see JOHNSON, 11)
      Hybrids, *Stiporyzopsis*
1. First glume shorter than or equal to lemma (longer than lemma in *O. micrantha*), some glumes only 1-nerved in some species, equal, or the second exceeding the first
  Section *Euoryzopsis*
  6. Lemma 2–2.5 mm. long, glabrous or rarely pubescent................*O. micrantha*
  6. Lemma more than 3 mm. long
    7. Lemma pubescent, at least at base, pale
      8. Lemma pubescent throughout, 3.5–5 mm. long
        9. Awn less than 2 mm. long
          O. pungens
        9. Awn 5 mm. long or more
          10. Panicle branches spreading
            O. canadensis
          10. Panicle branches erect
            11. Awn about 5 mm. long, sharply once geniculate
              O. exigua
            11. Awn about 10 mm. long, curved or weakly geniculate..........*O. kingii*

  8. Lemma densely pubescent on callus, nearly glabrous above, about 6 mm. long...............*O. asperifolia*
  7. Lemma glabrous, brown at maturity
    O. hendersoni

Natural affinities in Oryzopsis

I. DIPLOID SPECIES OF SECTION PIPTATHERUM

Five Old World species of *Oryzopsis* included in this study—*O. miliacea* (L.) Benth. and Hook., *O. virescens* (Scop.) Beck., *O. paradoxa* (L.) Nutt., *O. coerulescens* (Desf.) Hack., and *O. holciformis* (Bieb.) Hack.—were placed by TRINIUS and RUPRECHT (18) in the section *Piptatherum* of the genus *Urachne*. They are here referred to the section *Piptatherum* under *Oryzopsis* and are treated as diploids on the basis of chromosome counts for three of them. The two species of unknown chromosome numbers are included because of their obvious close affinity on morphological characters to the other three. These five species range from southern Europe and northern Africa to Asia Minor and northeastward into Asiatic Russia. While they show variation in the size and number of spikelets and in the density of the panicle, they nevertheless are a distinctly homogeneous group whose coherence is traceable through characters of the floral parts, inflorescence, and to some extent in vegetative characters (figs. 1–12; tables 1, 2).

It will be noted (table 1) that while the lemma is greatly variable in size, from a length of about 2 mm. in *O. miliacea* (fig. 1) to about 7 mm. in *O. holciformis* (fig. 10), the species figured here still shows a relationship in its general features. The lemma varies slightly in outline (table 2), from obovate in *O. miliacea* and *O. virescens* to elliptic in *O. paradoxa*, *O. coerulescens*, and *O.*
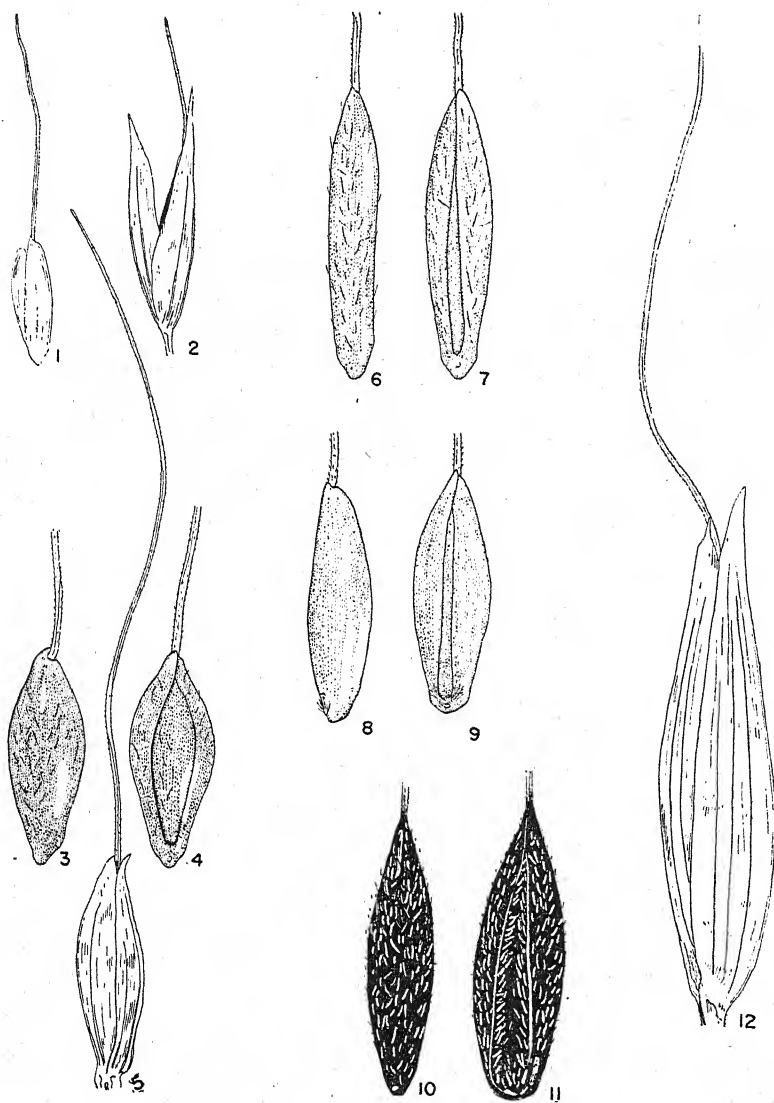
FIGS. 1–12.—Floral parts: Figs. 1, 2, *Oryzopsis miliacea;* fig. 1, lemma; fig. 2, spikelet. Figs. 3–5, *O. virescens;* fig. 3, lemma side view; fig. 4, lemma front view; fig. 5, spikelet. Figs. 6, 7, *O. paradoxa;* fig. 6, lemma side view; fig. 7, lemma front view. Figs. 8, 9, *O. coerulescens;* fig. 8, lemma side view; fig. 9, lemma front view. Figs. 10–12, *O. holciformis;* fig. 10, lemma side view; fig. 11, lemma front view; fig. 12, spikelet. All ×10.

holciformis. It is quite uniform throughout this group in lacking a clearly differentiated callus. In the case of *O. coerulescens* (fig. 8) and *O. holciformis* (fig. 10), the basal end of the floret is reduced rather than modified to form a callus, as in the other sections of the genus. In *O. miliacea* the edges of the lemma form

TABLE 1

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE
PARTS OF FIVE SPECIES OF ORYZOPSIS OF SECTION PIPTATHERUM

	O. miliacea	O. virescens	O. paradoxa	O. coerulescens	O. holciformis
Pedicels:					
Longer.............	3.1±0.3	15.0±6.5	8.9±1.1	4.3±1.9	10.4±4.9
Shorter............	1.2±0.3	10.9±5.3	4.4±1.1	1.6±1.2	5.0±2.5
Glumes:					
First..............	3.6±0.4	4.6±0.3	6.5±0.7	7.8±1.1	10.2±2.0
Second............	3.2±0.3	4.4±0.2	5.4±0.7	7.1±0.7	9.7±1.9
Lemma..............	2.2±0.2	3.6±0.2	4.2±0.3	4.3±0.3	6.6±1.1
Hairs of lemma.......	None	0.3±0.0	0.3±0.0	None	0.3±0.1
Palea...............	1.9±0.2	3.1±0.1	3.6±0.3	3.9±0.3	6.0±1.0
Anthers:					
Longest...........	1.4±0.1	2.3±0.1	2.6±0.2	2.9±0.3	4.0±0.9
Next to longest.....	1.4±0.1	2.3±0.1	2.6±0.2	2.9±0.3	4.0±0.8
Anther beards........	0.1+0.0	0.1±0.0	None	0.1±0.0	Rudimentary
Awn................	3.7±0.7	12.6±1.1	11.7±2.2	3.1±1.1	10.3±3.2
Ligule..............	1.7±0.6	0.8±0.3	0.7±0.1	7.0±2.2	8.5±2.1
No. of measurements..	10	10	4	10	10

TABLE 2

MORPHOLOGICAL CHARACTERS OF FIVE SPECIES OF ORYZOPSIS OF SECTION PIPTATHERUM

	O. miliacea	O. virescens	O. paradoxa	O. coerulescens	O. holciformis
Panicle..............	Open	Open	Open	Open	Open
Glumes:					
Shape.............	Acuminate	Acute	Acuminate	Acuminate	Acuminate
No. of nerves—					
First glume.......	3	3 (5)*	5	5–9	5–8
Second glume.....	3	3 (5)	3	5–7	5–7
Lemma:					
Shape.............	Obovate	Obovate	Elliptic, flat	Elliptic, flat	Elliptic, flat
No. of nerves......	3	5 (7)	5 (6)	5 (6)	5
Summit...........	Minutely lobed	Lobed	Short lobed	Minutely lobed	Not lobed
Palea:					
Shape.............	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
No. of nerves......	2	2	2 (4)	2	2
Anther beards per sac..	3–8	0–4	0	0–5	0–7
Awn:					
Persistence........	Deciduous	Deciduous	Deciduous	Deciduous	Tardily deciduous
Geniculation.......	Straight	Flexuous	Flexuous	Straight	Flexuous
Texture...........	Scaberulous	Scaberulous	Scaberulous	Scaberulous	Scaberulous
Throat of sheath......	Glabrous	Sparsely pubescent	Sparsely pubescent	Glabrous	Glabrous
Pollen..............	Normal	Normal	Normal	Normal	Normal

* Numbers in parentheses represent a few exceptional cases.

two small lobes in front of the awn. In *O. virescens* these lobes are more distinct, but in the other species they are reduced, being absent in *O. holciformis*. Together with this reduction of the callus and the lobes, there is a correlated dorsiventral flattening and induration of the lemma. All these features seem to be further correlated with increase in size. The small, thin lemma of *O. miliacea* also has only three nerves, while the larger, indurate lemmas of the other species considered here have from five to seven nerves. The lemma is typically glabrous or sparsely short pubescent, varying from pale in *O. miliacea* to dark brown in *O. virescens*, *O. paradoxa*, and *O. coerulescens*, and black in *O. holciformis*.

The awn in all these species is weak. In all of them except *O. holciformis* it separates readily at the point of articulation with the lemma. Such a point of articulation is very obscure in *O. holciformis*, and the awn is tardily deciduous, usually breaking off and leaving a jagged edge. *O. coerulescens* has a very much reduced and early deciduous awn. It is shorter than the body of the lemma. This reduction and early deciduousness of the awn occur sporadically in other groups of the Stipeae. Care should therefore be used in attaching any taxonomic significance to it beyond its specific value in these cases.

The anthers of *O. miliacea* are bearded at the apex. In the rest of the species the beards are much fewer. In *O. holciformis* they are rudimentary. None were found in the small amount of material examined of *O. paradoxa*.

A consistent character for the species of this section is found in the style branches (fig. 13), which are reflexed at anthesis, emerging some distance below the summit of the lemma.

In all the Old World species the first glume is longer than the second, and both of them distinctly exceed the lemma (tables 1, 3). They vary from 3-nerved in *O. miliacea* to 8- or 9-nerved in *O. coerulescens* and *O. holciformis*. As compared with other sections, the glumes in this section are somewhat inflated, loosely investing the lemma.

The panicle is open and characterized by distant, spreading branches which bear
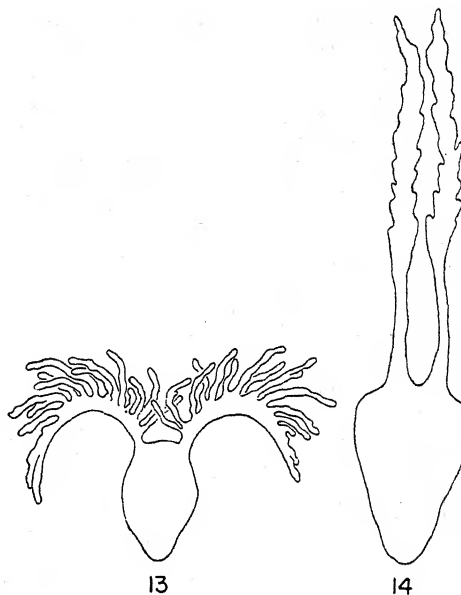
FIGS. 13, 14.—Style branches: Fig. 13, *Oryzopsis miliacea*. Fig. 14, *O. micrantha*. ×40.

their spikelets distally. In *O. miliacea* the branches are several at each node. In *O. virescens* they are much fewer, and the branches and pedicels are long and capillary, producing a somewhat diffuse panicle. *O. coerulescens* and *O. holciformis* bear the panicle branches mostly singly or in pairs at the nodes and have shorter pedicels than has *O. virescens*.

The leaves in this section are typically broad and flat, the uppermost ones on the culms frequently being reduced in length. This reduction occurs also in the other

sections of the genus. The culms of *O. miliacea* have several nodes, while in the rest of the species the nodes are three

or less per culm, as in other sections of *Oryzopsis*.

Of the various species in the section *Piptatherum*, *O. virescens* is most nearly representative of the entire genus *Oryzopsis* on a number of characters. It is not excessively specialized in those characters which are peculiar to the section *Piptatherum*. From the condition found in this species, specialization within the section *Piptatherum* involves some reduction of the callus, greater induration and dorsiventral flattening of the lemma and increase in its size, together with an increase in length and nervation of the glumes. *O. holciformis* is in these respects the most highly specialized species in this section. *O. miliacea* resembles the more highly specialized species in the shape and relative lengths of the glumes, but the small size of its floral parts and the lack of induration of its lemma suggest reduction. Also it departs from sectional characters in type of panicle and number of nodes per culm. Characters approaching those of *O.*

TABLE 3

RATIOS OF AVERAGE GLUME LENGTH TO LEMMA LENGTH AND OF LENGTH OF FIRST TO LENGTH OF SECOND GLUME IN ORYZOPSIS

Species	Av. glume / Lemma	Glume 1 / Glume 2
Diploid species:		
Section Piptatherum—		
O. miliacea.........	1.55	1.12
O. virescens.........	1.25	1.05
O. paradoxa*.......	1.42	1.20
O. coerulescens*......	1.73	1.09
O. holciformis........	1.51	1.05
Section Euoryzopsis—		
O. micrantha........	1.41	0.97
O. pungens..........	0.88	1.03
O. canadensis*.......	1.10	0.97
O. exigua*..........	0.95	0.93
O. kingii...........	1.09	0.85
Polyploid species:		
Section Piptatherum—		
O. racemosa........	1.14	1.00
Section Euoryzopsis—		
O. asperifolia........	1.04	0.96
O. hendersoni*.......	1.06	0.98

*Chromosome numbers not known.

TABLE 4

CHROMOSOME NUMBERS IN SPECIES OF ORYZOPSIS AND STIPA

SPECIES	NUMBER		PREVIOUS RECORD	
	n	2n	2n	Authority
Section Piptatherum:				
O. miliacea..............	12	24	24	Avdulov, 1928
O. virescens.............			24	Avdulov, 1928
O. holciformis...........		24		
O. racemosa.............		46		
Section Euoryzopsis:				
O. micrantha............		22		
O. pungens.............		22		
O. kingii...............		22		
O. asperifolia...........		46		
Section Eriocoma:				
O. hymenoides..........	24	48		Stebbins and Love, 1941
Genus Stipa:				
S. webberi.............	16	32		
S. pinetorum............			32	Stebbins, unpublished
S. sibirica..............	12	24	24	Avdulov, 1928

virescens, which appears to show neither excessive specialization nor reduction, would serve not only as a possible point of divergence for the other species of the section *Piptatherum* but also as a plausible source of characters for the sections *Euoryzopsis* and *Eriocoma*. That the section *Piptatherum* may be ancestral to both of the other sections, at least in part, is indicated by chromosome numbers.

The differentiation of such diverse species as *O. miliacea* and *O. holciformis* in the section *Piptatherum* has been accomplished with scarcely any detectable alteration in the karyotype. AVDULOV (1) reports somatic complements of 24 chromosomes for *O. miliacea* and *O. virescens*. The count for *O. miliacea* has been verified in this study (table 4). In addition, original counts for *O. holciformis* made in this study show that it also has 2n = 24 chromosomes (table 4). The metaphase figures of *O. miliacea* and *O. holciformis* (figs. 15, 16) show no great differences. The chromosomes, as compared with other species of *Oryzopsis*, are small, both narrower and shorter. In each case two chromosomes of about average length bear satellites. In the particular figure of *O. holciformis* shown in figure 16 only one satellite was visible.

Idiograms (figs. 24, 25) of a chromosome set of each species based on three somatic metaphase figures show the chromosomes of both species to vary in length from almost 2μ to less than 1μ. The satellite is associated with a chromosome of approximately the same length in each case. It is doubtful whether much significance can be attached to minor variations within the set as revealed by these idiograms. In *O. holciformis*, however, the first or longest chromosome is a third again as long as the second.

The count of 2n = 24 chromosomes

for *O. holciformis* obtained in this study adds further evidence to that already cited by AVDULOV (2) to the effect that the basic haploid number in *Oryzopsis*
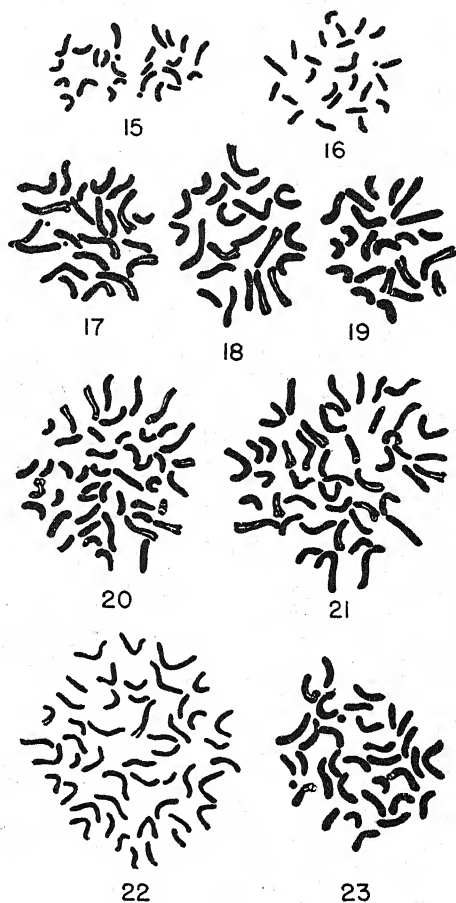
FIGS. 15–23.—Metaphase chromosomes from root tips: Fig. 15, *Oryzopsis miliacea*, 2n = 24. Fig. 16, *O. holciformis*, 2n = 24. Fig. 17, *O. micrantha*, 2n = 22. Fig. 18, *O. pungens*, 2n = 22. Fig. 19, *O. kingii*, 2n = 22. Fig. 20, *O. racemosa*, 2n = 46. Fig. 21, *O. asperifolia*, 2n = 46. Fig. 22, *O. hymenoides*, 2n = 48. Fig. 23, *Stipa webberi*, 2n = 32. All × 2420. (Fig. 22 redrawn from JOHNSON and ROGLER, Amer. Jour. Bot.)

is 12. AVDULOV (1) also reports a count of 2n = 24 for *Stipa sibirica* Lam. and concludes (2) that 12 is the basic haploid number for that genus as well. His count for *S. sibirica* is verified in this study by

several counts of n = 12 obtained at diakinesis.

The species of the section *Piptatherum* considered here are treated as diploids on the basis of x = 12, although evidence to be discussed later indicates that the primary basic number for both *Stipa* and *Oryzopsis* possibly is x = 6, as suggested by STEBBINS and LOVE (**16**).

The following herbarium specimens[1]

[1] The herbaria from which specimens were loaned are designated by letters as follows: University of California, Berkeley (UC); United States National Museum (US); Missouri Botanical Garden (MBG); University of Idaho, Southern Branch, Pocatello
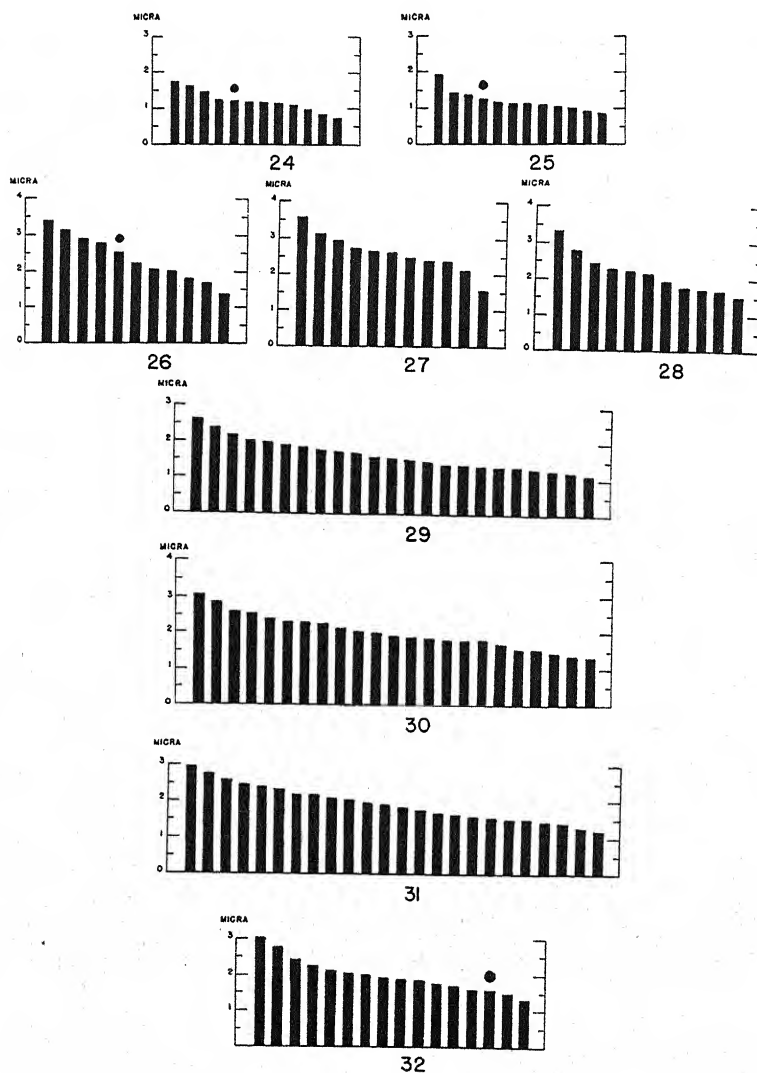
FIGS. 24–32.—Idiograms of chromosomes at somatic metaphase: Fig. 24, *Oryzopsis miliacea*. Fig. 25, *O. holciformis*. Fig. 26, *O. micrantha*. Fig. 27, *O. pungens*. Fig. 28, *O. kingii*. Fig. 29, *O. racemosa*. Fig. 30, *O. asperifolia*. Fig. 31, *O. hymenoides*. Fig. 32, *Stipa webberi*.

were used in compiling the data for tables 1 and 2:

Oryzopsis miliacea.—France: Bellevue, 1902, Bertrand (UM); Annot, 1902, Legrelle (UM). Canary Islands: Cook 739 (MBG). Palestine: Jerusalem, Dinsmore and Meyers 2146a (UM). Tunisia: Gabes, Pitard 288 (MBG).

Oryzopsis virescens.—Rumania: "Ad thermas Herkulis," Thaisz 15 (UM); 1923, Borza and Nyarady (MBG); between Plavisevita and Dubova, 1910, Peterfi (MBG). Hungary: Buda (MBG 210644). Yugoslavia: Gottschee, 1826, Müller (MBG).

Oryzopsis paradoxa.—Algeria: Tlemcen, 1856 (MBG 210637); 1906–07, Gandoger (MBG).

Oryzopsis coerulescens.—Greece: Macedonia (MBG 210619). Turkey: Smyrna, 1827, Fleischer (MBG). Sicily: Pisano (MBG 210617). "Rupibus Montis Catharinae" (probably Mt. Sinai): 1835, Schimper (MBG). France: Aude, La Nouvelle, 1901, Sennen (UM).

Oryzopsis holciformis.—Rumania: Orsova, Degen 3991 (MBG); 1910, Peterfi (MBG); between Plavisevita and Dubova, Degen 16 (UM). Palestine: Jerusalem, Meyers and Dinsmore 2687 (UM). Persia: Shiraz, 1845, Hohenacker (MBG).

II. DIPLOID SPECIES OF SECTION EUORYZOPSIS

Seven North American species of *Oryzopsis* are included in the section *Euoryzopsis*. Five of these—*O. micrantha* (Trin. and Rupr.) Thurb., *O. pungens* (Torr.) Hitchc., *O. canadensis* (Poir.) Torr., *O. exigua* Thurb., and *O. kingii* (Boland.) Beal—form a basal group in that section and are here treated as dip-

(UIS); University of Minnesota (UM); State College of Washington, Pullman (WS); University of Wyoming (UWy); University of Colorado (UCo).

loids, on the basis of chromosome counts for three of them and on the strength of morphological similarity on the part of the other two species. TRINIUS and RUPRECHT (18) included *O. micrantha* in their section *Oryzopsis*, which was about equivalent to the present section *Euoryzopsis*, at the same time pointing out its close affinity to *O. miliacea*—which they placed in the section *Piptatherum*. *O. pungens* (Torr.) Hitchc., which was originally described under the generic name of *Milium*, was included by TRINIUS and RUPRECHT in their section *Oryzopsis* as a synonym of *O. canadensis* (Poir.) Torr. HITCHCOCK (7) recognized them as distinct species. *O. canadensis* and *O. kingii* were originally described under *Stipa*. *O. exigua* is the only one of these five species originally described under its present generic name. While this group of species is coherent on a number of characters, *O. micrantha* is somewhat detached from the rest by its affinity to the section *Piptatherum*.

In contrast to the section *Piptatherum*, this group of species (figs. 33–42; tables 5, 6) shows no induration nor dorsiventral flattening of the lemma, nor any appreciable variation in its size. The lemma in *O. micrantha* (fig. 33) is obovate and mostly glabrous, although a less common form has a pubescent lemma. In the other four species it is pubescent and fusiform, attaining a narrow, stipoid form in *O. kingii* (fig. 41). In this group, departure from the characters of the genus as a whole is expressed in greater differentiation of a callus and the development of a strong, twisted awn, which in some species is geniculate and persistent. These are the features which in their more extreme expression constitute the generic characters of *Stipa*. In fact, on the basis of morphological characters there is no definite line of

demarcation between *Stipa* and this section of *Oryzopsis*.

In *O. micrantha* the callus is weakly developed, as in the section *Piptatherum*. In *O. pungens* (fig. 35) it is blunt and slightly swollen, while in *O. exigua* (fig. 37), *O. canadensis* (fig. 39), and *O. kingii*

it is longer and more clearly differentiated from the body of the lemma. In *O. kingii* it is as sharp as in some species of *Stipa*.

The awn in *O. micrantha* is weak and flexuous, as in the section *Piptatherum*. In *O. pungens* it is greatly reduced, but
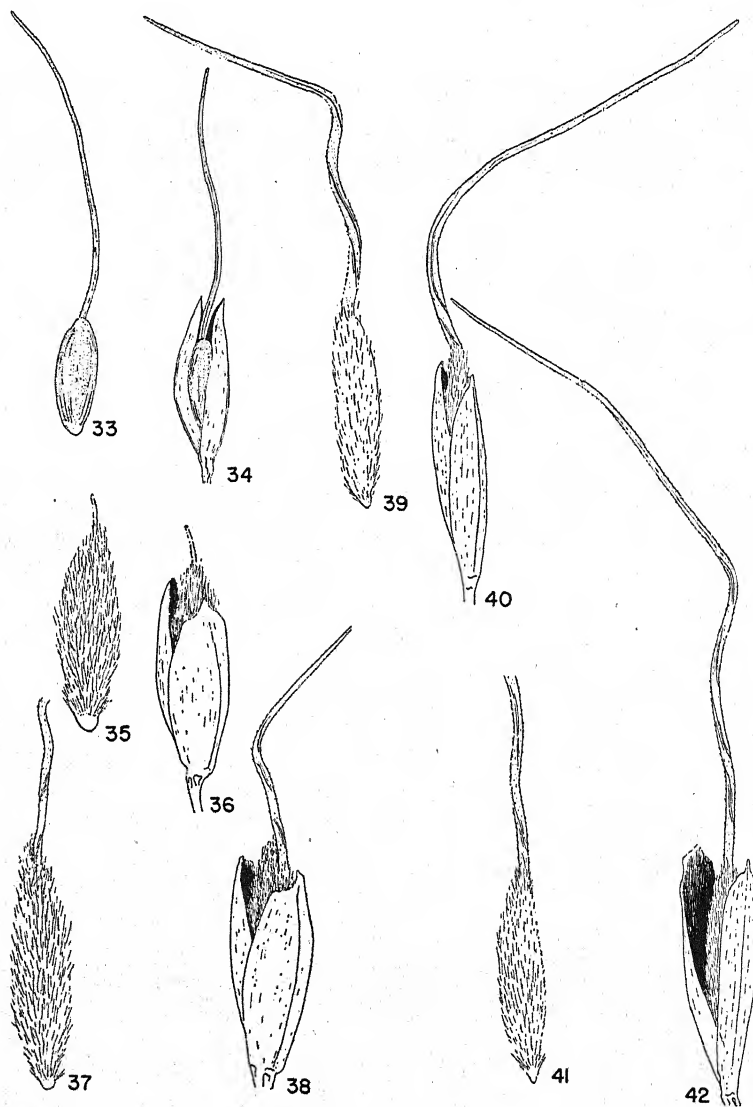
FIGS. 33–42.—Floral parts: Figs. 33, 34, *Oryzopsis micrantha;* fig. 33, lemma; fig. 34, spikelet. Figs. 35, 36, *O. pungens;* fig. 35, lemma; fig. 36, spikelet. Figs. 37, 38, *O. exigua;* fig. 37, lemma; fig. 38, spikelet. Figs. 39, 40, *O. canadensis;* fig. 39, lemma; fig. 40, spikelet. Figs. 41, 42, *O. kingii;* fig. 41, lemma; fig. 42, spikelet. All ×10.

such an extreme expression of this character apparently has no significance beyond the species in which it occurs, as previously pointed out. In *O. exigua* the awn is about 7 mm. long, sharply once geniculate, and twisted. In *O. canadensis* it is longer, twisted, and obscurely twice geniculate. In *O. kingii* the awn reaches

TABLE 5

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE
PARTS OF FIVE SPECIES OF ORYZOPSIS OF SECTION EUORYZOPSIS

	O. micrantha	O. pungens	O. canadensis	O. exigua	O. kingii
Pedicels:					
Longer............	3.1±0.6	5.8±1.3	7.7±1.9	7.0±1.7	4.4±0.8
Shorter...........	1.4±0.4	3.0±0.9	4.3±2.0	2.4±1.3	1.8±0.6
Glumes:					
First.............	3.2±0.3	3.3±0.5	3.8±0.2	4.2±0.5	3.5±0.5
Second...........	3.3±0.3	3.2±0.1	3.9±0.1	4.5±0.5	4.1±0.5
Lemma.............	2.3±0.2	3.7±0.2	3.5±0.3*	4.6±0.4	3.5±0.3
Hairs of lemma......	None	0.4±0.0	0.5±0.0*	0.4±0.1	0.4±0.0
Palea..............	2.0±0.2	3.4±0.2	2.9±0.3*	4.3±0.5	2.7±0.2
Anthers:					
Longest..........	1.1±0.1	2.1±0.2	2.0±0.1*	2.4±0.3†	1.7±0.2
Next to longest.....	1.1±0.1	2.1±0.2	2.0±0.1*	2.4±0.3†	1.7±0.1
Anther beards........	None	Rudimentary	0.1±0.0	0.5±0.2	0.1±0.7
Awn................	6.5±1.4	1.2±0.2	8.5±0.8	5.6±1.1	11.1±1.7
Ligule.............	1.7±0.3	1.8±0.5	1.9±0.5	3.0±0.7	2.0±0.4
No. of measurements..	22	16	8	16	16

* Based on 6 measurements.

† Based on 13 measurements.

TABLE 6

MORPHOLOGICAL CHARACTERS OF FIVE SPECIES OF ORYZOPSIS OF SECTION EUORYZOPSIS

	O. micrantha	O. pungens	O. canadensis	O. exigua	O. kingii
Panicle..............	Open	Mostly narrow	Open	Narrow	Narrow
Glumes:					
Shape.............	Acuminate	Obtuse or acute	Obtuse or acute	Obtuse or acute	Obtuse
No. of nerves—					
First glume.......	1–3 (5)*	1–3 (5)	1–3	1–3 (5)	0–1 (3)
Second glume.....	1–3 (5)	1–3 (5)	1–3 (5)	1–3 (5)	1 (3)
Lemma:					
Shape.............	Obovate	Fusiform	Fusiform	Fusiform	Fusiform
No. of nerves.......	5	5 (7)	5	5 (7)	3 (5)
Summit...........	Not lobed	Minutely lobed	Not lobed	Lobed	Minutely lobed.
Palea:					
Shape.............	Broad elliptic	Elliptic	Elliptic	Elliptic	Elliptic
No. of nerves.......	2	2	2	2 (4)	2
Anther beards per sac..	0–1 (2)	0 (2)	0–4	0–3 (5)	3–7
Awn:					
Persistence........	Deciduous	Deciduous	Tardily deciduous	-Tardily deciduous	Mostly persistent
Geniculation........	Flexuous	Straight	Twice (weakly)	Once	Curved
Texture...........	Scaberulous	Scaberulous	Scaberulous	Scaberulous	Scaberulous
Throat of sheath......	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Pollen..............	Normal	Normal	Normal	Normal	Normal

* Numbers in parentheses represent a few exceptional cases.

its greatest development and is twisted and weakly geniculate. In *O. micrantha* and *O. pungens* the awn is early deciduous. In *O. exigua* and *O. canadensis* it is deciduous at maturity, but in *O. kingii* it is often quite persistent, as in the genus *Stipa*. Thus the characters which are usually relied upon to separate the two genera break down within this group of stipoid species of *Oryzopsis*.

In the section *Euoryzopsis* the second glume is longer than the first, except in *O. pungens* (tables 3, 5). In *O. micrantha* (fig. 34; table 6) the glumes are acuminate and exceed the lemma. In this respect they resemble the section *Piptatherum*. However, they are thin and frequently only 1-nerved, suggesting the reduction of the glumes—which is more clearly expressed in the other four species of the section *Euoryzopsis* considered here. These species (figs. 36, 38, 40, 42; tables 5, 6) show reduction in nervation and length of glumes to the condition found in *O. kingii*, which has obtuse, mostly 1-nerved glumes that are shorter than the lemma.

The panicle in *O. micrantha* is open, often with reflexed branches similar to that of the section *Piptatherum*. In *O. pungens* it is mostly narrow but sometimes open, as it is also in *O. canadensis*. In *O. exigua* and *O. kingii* it is narrow, as in the genus *Stipa*. The anthers of these species are mostly beardless, or have only a few small beards, as in the section *Piptatherum*. *O. kingii* has three to seven beards per anther sac. The leaves in all these species are narrow and in most of them involute, in contrast to the wide, flat leaves of the section *Piptatherum*.

Another feature which appears effectively to separate these two groups is the style branches, which in the section *Euoryzopsis* (fig. 14) are erect at anthesis, emerging at the summit of the lemma,

while in the section *Piptatherum* (fig. 13) they are reflexed, emerging below the summit.

O. pungens and *O. canadensis* are close together taxonomically, differing mostly in the character of the awn. They also occupy similar ranges (figs. 44, 46), with *O. pungens* more widely distributed in the United States. Authentic material of *O. canadensis* is scarce. Only four collections, ranging from northern Labrador to the New England states and Michigan, have been examined in this study. The range of *O. pungens* extends westward in United States and Canada, probably coming in contact with the range of *O. exigua* (fig. 45). On the other hand, *O. kingii* (fig. 47) is endemic to the meadows at high altitudes in the central Sierra Nevada Mountains, where it occurs abundantly. The geographic range of *O. micrantha* (fig. 43) is roughly similar to that of *O. exigua*.

Thus, while this group of species is coherent on a number of characters, it is apparent that at the more specialized extreme *O. kingii* intergrades with the genus *Stipa*, while at the less specialized extreme *O. micrantha* shows an admixture of characters of *Piptatherum*. *O. kingii*, which was transferred from *Stipa*, is so closely allied to that genus on most characters that its assignment to either *Oryzopsis* or *Stipa* is largely arbitrary. Its narrow, non-indurate lemma, with a sharp callus and strong, twisted, persistent awn, its narrow panicle, and slender, involute leaves are characters which would place this species in *Stipa* were it not for the gradation toward *Oryzopsis* supplied by the other stipoid species. The affinity of *O. micrantha* to the section *Piptatherum* is recognized by ELIAS (**5**), who placed it in that section. The admixture of *Piptatherum* characters in *O. micrantha* is evidenced by its flexuous

awn and weak callus, its open panicle with reflexed branches, and its acuminate glumes. In these features *O. micrantha* is abruptly set off from the other four stipoid species in the section *Euoryzopsis* considered here.

Aside from these characters, similarity between *O. miliacea* and *O. micrantha* appears to be mostly superficial. The lemmas of both are of about the same length and smaller than in any other species of their respective sections. This apparent
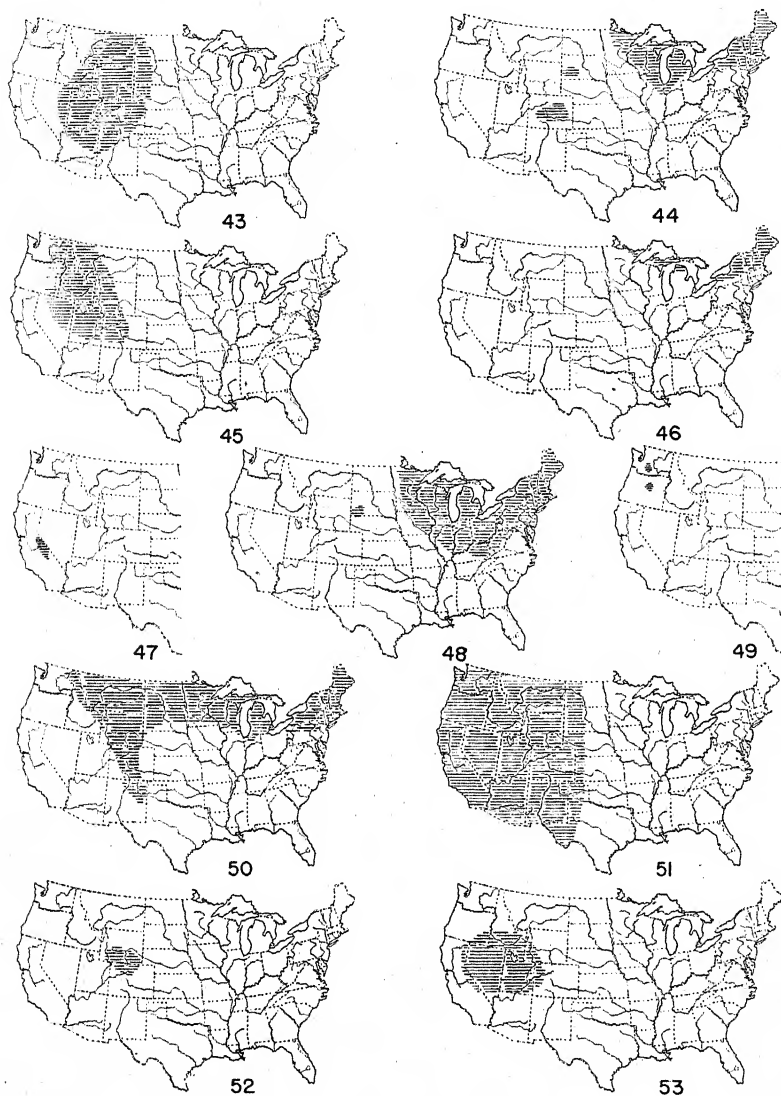
FIGS. 43–53.—Geographic distribution of North American species of *Oryzopsis* and *Stipa webberi:* Fig. 43, *O. micrantha*. Fig. 44, *O. pungens*. Fig. 45, *O. exigua*. Fig. 46, *O. canadensis*. Fig. 47, *O. kingii*. Fig. 48, *O. racemosa*. Fig. 49, *O. hendersoni*. Fig. 50, *O. asperifolia*. Fig. 51, *O. hymenoides*. Fig. 52, *O. hymenoides* var. *contracta*. Fig. 53, *Stipa webberi*.

reduction has left the glumes of *O. micrantha* longer than the lemma, simulating the condition in *O. miliacea*, but other features of the glumes in the former species still show its sectional affinity. The second glume exceeds the first, and both show the reduction in nervation characteristic of the section *Euoryzopsis*. The major affinity of *O. micrantha* to that section is further evidenced by its narrow, involute leaves, its erect style branches, and its geographic range. To this may be added the evidence from chromosome numbers. Thus, by virtue of the combination of characters in *O. micrantha*, the other four stipoid species in the section *Euoryzopsis* articulate with the section *Piptatherum*.

O. micrantha was found to have a somatic complement of 22 chromosomes (table 4). As in the Old World species, many figures (fig. 17) were observed in which two chromosomes of about average length bore satellites. The chromosomes, however, are much larger than in either *O. miliacea* or *holciformis*. From the idiogram (fig. 26) it will be noted that they range in length from about 3.5 to 1.5μ, most of them being longer than the longest chromosomes in the Old World species. *O. pungens* and *O. kingii* were also found to have 2n = 22 chromosomes (figs. 18, 19), with a similar range in length (figs. 27, 28). Cytological material was not obtained for *O. exigua* and *O. canadensis*.

On the basis of karyotype, therefore, *O. micrantha*, *O. pungens*, and *O. kingii* are quite similar, and, except for the combination of characters in *O. micrantha*, the sections *Piptatherum* and *Euoryzopsis*, as judged from the species thus far considered, appear to have diverged along separate lines of specialization, having basic chromosome numbers of x = 12 (or 6) and x = 11, respectively.

Within the section *Piptatherum*, as previously pointed out, specialization involves reduction of the callus, induration and dorsiventral flattening of the lemma and increase in its size, together with an increase in length and nervation of the glumes. Within the section *Euoryzopsis*, *O. micrantha* presents some *Piptatherum* characters, but the rest of this line with x = 11 chromosomes illustrates specialization of the awn and callus and the development of a stipoid lemma, together with reduction in length and nervation of the glumes. This line evidently terminates in the genus *Stipa*, where it is represented by several polyploid species.

Avdulov (2) reports counts of 2n = 44 chromosomes in seven Old World species of *Stipa* and concludes that, in addition to the primary basic number of x = 12, a secondary basic number of x = 11 has arisen in that genus. Nielsen (13), working with American species, lists 2n = 44 chromosomes for *Stipa columbiana* Macoun and 2n = 66 for *S. pulchra* Hitchc. Further evidence for the existence of two basic numbers in *Stipa* is supplied by Stebbins and Love (16), who report aneuploidy in that genus, with chromosome counts which suggest a modified polyploid series on the basis of x = 6 and x = 11. It seems probable that the line tentatively isolated by the change to x = 11 chromosomes has evolved through less specialized forms, such as the stipoid species in the section *Euoryzopsis*, and finally has become highly specialized in tetraploid and hexaploid species of *Stipa*. The x = 12 (or 6) line, as previously mentioned, is represented in *Stipa* by *S. sibirica* with 2n = 24 chromosomes, and —as pointed out by Stebbins and Love (16)—probably also by a number of American species which have 2n = 36

chromosomes. The two basic lines in that genus, however, become obscured by the conspicuous aneuploid series. This series may be in part the result of allopolyploidy between the two lines.

The following herbarium specimens were used in compiling the data for tables 5 and 6:

Oryzopsis micrantha.—Alberta (Assiniboia): Medicine Hat, Macoun 7505 (UM). North Dakota: Morton County, Colebank 77 (UM). South Dakota: Harney Peak, 1924, McIntosh (UWy). Montana: Lower Sand Coulee, Williams 815 (UM). Nebraska: War Bonnet Canyon, 1890, Williams (UM). Wyoming: New Castle, Pammel 147 (UM). Utah: Fish Lake, Jones 5746 (UWy). Colorado: Larimer County, Gooding 1920 (UCo). Nevada: Deer Creek, Clokey 7825 (UM). New Mexico: Lincoln County, Earle and Earle 165 (UWy). Arizona: Flagstaff, MacDougal 296 (UWy).

Oryzopsis pungens.—Quebec: Calumet, 1891, Macoun (UM). New Hampshire: Carroll County, Weatherby and Smith 821 (UM). Vermont: Barnet, 1886, Blanchard (UM). Michigan: Keweenaw County, Hermann 7585 (UM). Wisconsin: Chippewa Falls, Rosendahl and Butters 3148 (UM). Minnesota: Lake County, Lakela 2404 (UM); Itasca Park, Nielsen 1935 (UM). British Columbia: Emerald Lake, Shaw 15 (UM).

Oryzopsis canadensis.—Labrador: Ungava River, Spreadborough 13345 (US). Maine: Aroostook County, St. John and Nichols 2123 (US). New Hampshire: Echo Lake, 1878 Faxon (US). Michigan: Marquette County, Hermann 7714 (US).

Oryzopsis exigua.—British Columbia: Coldwater River, Copley 56 (WS). Washington: Kititas County, Vasey 34 (WS). Oregon: Wallowa Mountains, Cusick 3302a (WS). Idaho: Custer

County, McBride and Payson 3430 (UWy), Nelson and McBride 1555 (WS). Montana: Yellowstone Falls, 1899 Blankenship (UWy). Wyoming: Lincoln County, Payson and Armstrong 3613 (UWy); Uinta County, Nelson and Nelson 6511 (UWy).

Oryzopsis kingii.—California: Mt. Silliman, 1905, Brandegee (UC); Fresno County, Hall and Chandler 601 (UM), Sharsmith 3082 (UC); Tuolumne County, Sharsmith 228 (UC), 1936, Hawbecker (UC); Mono County, Yates 6306 (UC); Mariposa County, 1898, Congdon (UM), 1894, Congdon (UM).

III. POLYPLOID SPECIES OF SECTIONS PIPTATHERUM AND EUORYZOPSIS

Three North American species of *Oryzopsis* characterized by large spikelets appear to combine some of the characters of the two lines represented by basic haploid chromosome numbers of $x = 11$ and $x = 12$ (or 6). These are *Oryzopsis racemosa* (J. E. Smith) Ricker, *O. asperifolia* Michx., and *O. hendersoni* Vasey. Chromosome counts were not obtainable for *O. hendersoni*. It is treated together with the other two species because it shows a similar combination of morphological characters.

In *O. racemosa* (figs. 54–56; tables 7, 8) the lemma is very similar to that in *O. holciformis*, being compressed dorsiventrally, greatly indurate, and black at maturity. As in that species also, the point of articulation of the awn at the summit of the lemma is obscure, and the awn is tardily deciduous—often breaking off and leaving an irregular edge. The awn is weak and flexuous, and the panicle is distinctly of the type described for the Old World species belonging to the section *Piptatherum*. The glumes are large and many-nerved, and the leaves are broad. However, the lemma in *O.*

racemosa is contracted near the base, forming an indistinct callus. A large scar is present at the point of union with the pedicel. These features suggest the section *Euoryzopsis*, in which the callus is well developed. The lemma is more pu-

bescent than is common in the section *Piptatherum*, and the glumes (table 3) are more nearly equal, more abruptly acuminate, and do not exceed the lemma as much as they commonly do in that section. These are characters which also
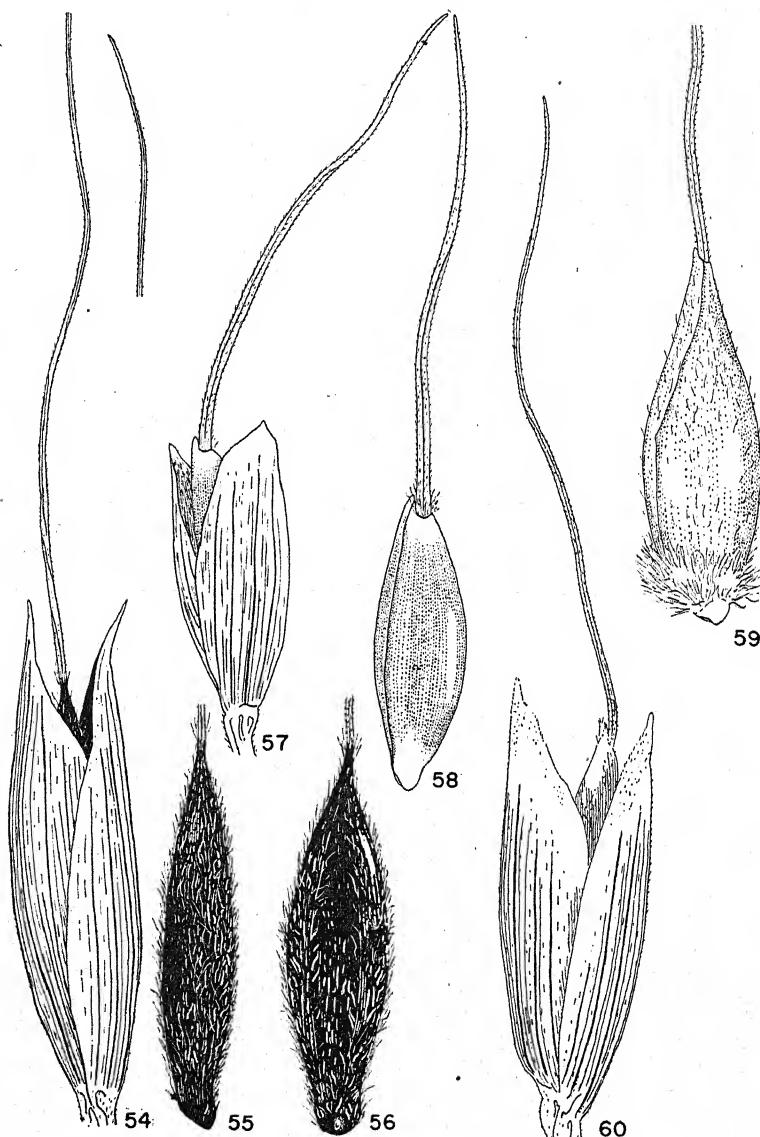
FIGS. 54–60.—Floral parts: Figs. 54–56, *Oryzopsis racemosa*; fig. 54, spikelet; fig. 55, lemma side view; fig. 56, lemma front view. Figs. 57, 58, *O. hendersoni*; fig. 57, spikelet; fig. 58, lemma. Figs. 59, 60, *O. asperifolia*; fig. 59, lemma; fig. 60, spikelet. All ×10.

suggest the section *Euoryzopsis*. This species was included in the section *Oryzopsis* by TRINIUS and RUPRECHT (18), but it is here included in the section *Piptatherum*—to which it seems more closely related on morphological char-

TABLE 7

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETA-
TIVE PARTS OF THREE SPECIES OF ORYZOPSIS OF SECTIONS
PIPTATHERUM AND EUORYZOPSIS

	O. racemosa	O. asperifolia	O. hendersoni
Pedicels:			
Longer.................	12.8±2.6	10.6±2.7	9.0±1.9
Shorter................	5.1±2.0	5.6±1.6	4.3±1.7
Glumes:			
First..................	8.0±0.5	6.6±0.7	4.5±0.3
Second................	8.0±0.5	6.9±0.7	4.6±0.3
Lemma.................	7.0±0.4	6.5±0.6	4.3*
Hairs of lemma...........	0.4±0.1	0.3±0.1	None
Palea	6.3±0.4	5.7±0.4	3.0*
Anthers:			
Longest................	4.0±0.4	3.7±0.4	....
Next to longest..........	4.0±0.4	3.7±0.4	....
Anther beards............	None	0.1±0.0	0.2*
Awn....................	18.3±1.7	10.1±1.6	9.0±0.5
Ligule.................	0.4±0.2	0.2±0.1	0.5*
No. of measurements........	24	24	5

* Based on single measurement.

TABLE 8

MORPHOLOGICAL CHARACTERS OF THREE SPECIES OF ORYZOPSIS
OF SECTIONS PIPTATHERUM AND EUORYZOPSIS

	O. racemosa	O. asperifolia	O. hendersoni
Panicle...................	Open	Mostly narrow	Narrow
Glumes:			
Shape.................	Abruptly acuminate	Abruptly acute	Abruptly acute
No. of nerves—			
First glume..........	7–9	7–9 (11)	5–7
Second glume........	(5) 7–9*	7–9	3–5
Lemma:			
Shape.................	Elliptic, flat	Broad fusiform	Broad fusiform
No. of nerves..........	5	7–9 (12)	5
Summit...............	Not lobed	Short lobed	Lobed
Palea:			
Shape.................	Elliptic	Elliptic	Elliptic
No. of nerves..........	2 or 4	2 (4)	2
Anther beards per sac......	0	20–40	3–5
Awn:			
Persistence...........	Tardily deciduous	Tardily deciduous	Deciduous
Geniculation...........	Flexuous	Curved	Curved
Texture...............	Scaberulous	Scaberulous	Puberulent
Throat of sheath..........	Puberulent	Glabrous	Glabrous
Pollen..................	Normal	Normal	....

* Numbers in parentheses represent a few exceptional cases.

acters. ELIAS (5) also included it in the latter section.

Oryzopsis racemosa was found to have 2n = 46 chromosomes (fig. 20). That number would be expected in an allotetraploid between a species with 2n = 24 and a species with 2n = 22 chromosomes. *O. racemosa* may represent such a tetraploid between the lines described for the sections *Piptatherum* and *Euoryzopsis*. The idiogram for this species (fig. 29) shows the chromosomes to range in length from about 2.6μ to about 1μ. This range takes in almost the entire range of *O. holciformis* plus much of the range of *O. micrantha*, for example. Thus, on the basis of chromosome number and length, together with features of gross morphology, *O. racemosa* seems to represent an allotetraploid between the two lines in *Oryzopsis* with basic numbers of x = 12 (or 6) and x = 11 (fig. 81). It is not presumed that any precise species of *Oryzopsis* still in existence were involved in this synthesis. On morphological characters, *O. racemosa* approaches most closely to *O. holciformis* on the *Piptatherum* side, but in present-day flora their geographic ranges are thoroughly isolated. The geographic range of *O. racemosa* (fig. 48) includes that of *O. pungens* and *O. canadensis*, at least in the United States.

O. asperifolia (figs. 59, 60; tables 7, 8) is the type of the section *Euoryzopsis*. On the majority of characters it is similar to the other species of that section, especially to *O. pungens*. It has a fusiform lemma with a swollen, blunt, pubescent callus. The glumes (table 3) are about equal to the lemma in length, and the second exceeds the first. The panicle is narrow, as is mostly the case in *O. pungens*. These two species are also similar in having the upper culm blades reduced to rudiments sometimes less than

5 mm. long. The basal leaves in both species are erect, firm, scabrous, and sharp-pointed. The geographic range of *O. asperifolia* (fig. 50) includes that of *O. pungens*, and their flowering season is the same—very early spring. These two species are, however, very different in size of plant as well as in size of floral parts, *O. asperifolia* being much the larger. In a few characters *O. asperifolia* shows affinity to the section *Piptatherum*. The lemma is more sparsely pubescent and more indurate than in the smaller species of the section *Euoryzopsis*. The awn is weak and flexuous, and the glumes are wide and many-nerved. These features are present in the section *Piptatherum*. The convolute, lobed lemma of *O. asperifolia* is found in only one other species, *O. hendersoni*.

O. asperifolia was also found to have 2n = 46 chromosomes (fig. 21), and may—like *O. racemosa*—represent an allotetraploid between the lines described for the sections *Piptatherum* and *Euoryzopsis* with species having 2n = 24 and 2n = 22 chromosomes, respectively. The idiogram (fig. 30) of *O. asperifolia* shows the chromosomes to range in length roughly from 3 to 1.5μ. That range includes only about three of the longest chromosomes in the idiograms of the diploid species illustrated from the section *Piptatherum* but includes most of the range in chromosome length of the diploid species illustrated from the section *Euoryzopsis*. There is no existing species in the section *Piptatherum* with 2n = 24 chromosomes whose geographic range is in contact with that of *O. asperifolia*, nor is it probable that the establishment of such an allotetraploid is of recent occurrence. The presence of *Piptatherum*-like species in the Tertiary flora of Colorado is evidenced by the work of ELIAS (5), who describes the fossil species

Paleoeriocoma hitchcocki from the Middle Pliocene and points out that it resembles the living species of the related section *Piptatherum* of the genus *Oryzopsis* in the dorsiventral flattening of the hull.

Oryzopsis hendersoni (figs. 57, 58; tables 7, 8) has been reported from only two localities, Mount Clements, Washington, and the Ochoco National Forest, Oregon (fig. 49). In size of plant and floral parts it is smaller than *O. asperifolia* or *O. racemosa*, but like these it seems to combine some of the characters of the more specialized 24- and 22-chromosome species in the sections *Piptatherum* and *Euoryzopsis*, respectively. The lemma is glabrous and dark brown, as in some species of the former section, but it is convolute as in *O. asperifolia* and has a moderately differentiated callus and a fairly strong awn, as in other species of the section *Euoryzopsis*. The wide several-nerved glumes suggest the section *Piptatherum*, but their length and the contracted panicle suggest the section *Euoryzopsis*. No cytological material was obtained for this species. It is included in the section *Euoryzopsis*.

The following herbarium specimens were used in compiling the data for tables 7 and 8:

Oryzopsis racemosa.—Maine: Auburn, 1898, Merrill (UM). Massachusetts: Woburn, 1890, Swan (UM). New York: Canandaigua Lake, 1892, Durand (UM). New Jersey: Sussex County, McKenzie 2308 (UM). Virginia: Giles County, Fogg 14914 (UM). Michigan: Hubbardston (without date), Wheeler (UM). Wisconsin: Devils Lake, 1896, Umbach (UM). Iowa: Ames, 1896, Ball (UM); 1897, Ball (UM). Minnesota: Becker County, Grant 3085 (UM); Hennepin County, 1889, Aiton (UM). South Dakota: Bigstone City, Moore 543 (UM).

Oryzopsis asperifolia.—Ontario: Edmonton, 1893, White (UM). Vermont: Peacham, 1888, Blanchard (UM). New York: Canandaigua, 1892, Durand (UM). Pennsylvania: Potter County, 1926, Sowden (UM). Michigan: Jackson County, 1897, Camp (UM). Wisconsin: St. Croix Falls, Benner 264 (UM). Minnesota: Carlton County, Lakela 1924 (UM). North Dakota: Rolette County, Lunell 873 (UM). South Dakota: Piedmont, 1895, Pratt (UM). Wyoming: Hulett County, Ownbey 598 (UWy). Washington: Metaline Falls, Hitchcock 2936 (WS). Colorado: Larimer County, 1897, Osterhout (UM).

Oryzopsis hendersoni.—Washington: Yakima County, Clements Mountain, Henderson 2249 Isotype (WS).

IV. SECTION ERIOCOMA

Oryzopsis hymenoides (Roem. and Schult.) Ricker (figs. 61-67; tables 9, 10), which belongs to the section *Eriocoma*, is characterized by a greatly indurate, broadly fusiform lemma (figs. 63, 66, 67) with a distinct, oblique callus and lobes at the summit. In its induration and somewhat in its outline it is similar to the lemma of *O. virescens* (figs. 3, 4), which belongs to the section *Piptatherum*, but the callus in *O. hymenoides* distinguishes it clearly. The lemma is clothed with long silky hairs almost equal in length to the lemma itself, and has a straight, early deciduous awn only slightly longer than the lemma. It has five nerves, with exceptional cases of three. The anthers are conspicuously bearded at the apex. The glumes are long acuminate and invest the lemma closely, the first being commonly 3-nerved and the second 5-nerved. The panicle is diffuse, with divaricate, long, capillary pedicels which are twice as long as the awned floret. These features also suggest the panicle of *O. virescens*.

Thus, in several features *O. hymenoides* shows affinity to the section *Piptatherum*, but it differs from the species of that section in the presence of a callus, in the long hairs of the lemma, and in the glumes which closely invest the lemma.

There is little if any resemblance to the section *Euoryzopsis* in these features, except in the callus. The glumes are even more narrowly acuminate than those in *Piptatherum* and differ greatly from the blunt glumes in the section *Euoryzopsis*.
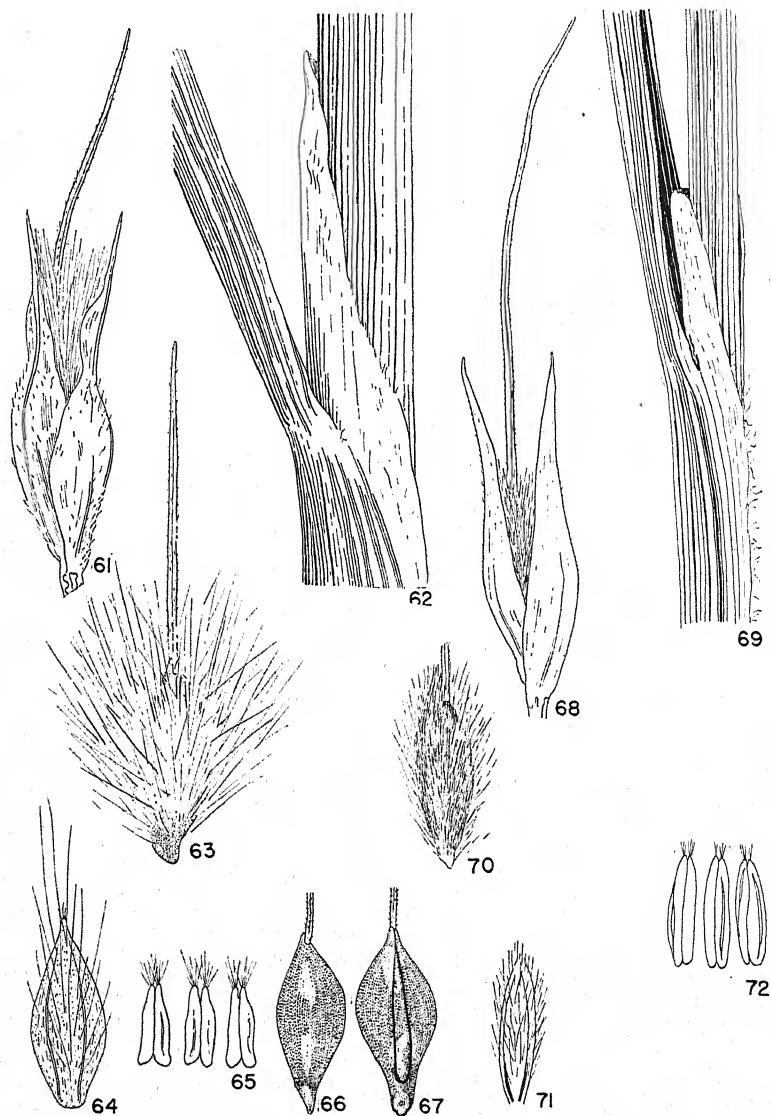
FIGS. 61–72.—Floral and vegetative parts: Figs. 61–67, *Oryzopsis hymenoides;* fig. 61, spikelet; fig. 62, ligule and throat of sheath; fig. 63, lemma; fig. 64, palea; fig. 65, anther set; fig. 66, lemma side view with hairs removed; fig. 67, same, front view. Figs. 68–72, *O. hymenoides* var. *contracta;* fig. 68, spikelet; fig. 69, ligule and throat of sheath; fig. 70, lemma; fig. 71, palea; fig. 72, anther set. All ×10.

TABLE 9

MEAN LENGTHS (WITH S.D.) IN MILLIMETERS OF FLORAL AND VEGETATIVE
PARTS OF ONE SPECIES AND A VARIETY OF ORYZOPSIS OF SECTION
ERIOCOMA AND OF TWO SPECIES OF STIPA

	O. hymenoides*	O. hymenoides var. contracta	S. webberi	S. pinetorum
Pedicels:				
Longer..................	14.7±4.4	7.8±2.2	8.4±2.5	6.0±0.9
Shorter..................	11.0±3.8	3.4±1.4	4.0±1.8	2.7±0.6
Glumes:				
First.....................	6.3±1.6	5.8±0.4	8.5±0.7	7.9±0.5
Second..................	5.6±0.8	5.9±0.3	8.5±0.7	7.8±0.5
Lemma.....................	3.3±0.3	3.2±0.2	5.5±0.5	4.5±0.1
Hairs of lemma.............	2.9±0.4	1.3±0.1	2.6±0.3	3.2±0.3
Palea.....................	2.8±0.3	2.9±0.2	4.7±0.3	2.5±0.1
Anthers:				
Longest..................	1.3±0.3	1.8±0.1‡	2.4±0.5	2.6±0.4
Next to longest...........	1.2±0.2	1.8±0.1‡	2.4±0.5	2.6±0.3
Anther beards..............	0.4±0.1	0.3±0.0‡	None	Occasional
Awn......................	4.5±1.1	7.4±0.7	6.3±0.9	12.4±0.8
Ligule....................	4.7±1.0†	4.5±1.0§	0.3±0.1	0.2±0.0
No. of measurements........	60	8	12	5

* Data for O. hymenoides from JOHNSON and ROGLER, Amer. Jour. Bot.

† Based on 43 measurements.   ‡ Based on 4 measurements.   § Based on 15 measurements.

TABLE 10

MORPHOLOGICAL CHARACTERS OF ONE SPECIES AND A VARIETY OF ORYZOPSIS
OF SECTION ERIOCOMA AND OF TWO SPECIES OF STIPA

	O. hymenoides*	O. hymenoides var. contracta	S. webberi	S. pinetorum
Panicle.....................	Diffuse	Contracted	Narrow	Narrow
Glumes:				
Shape....................	Ovate	Ovate	Lanceolate	Lanceolate
No. of nerves—				
First glume.............	3 (5)†	3 (+2 short)	3 (5)	3
Second glume..........	(3) 5 (7)	3 (5)	3 (5)	3
Lemma:				
Shape....................	Broad fusiform	Broad fusiform	Narrow fusiform	Narrow fusiform
No. of nerves.............	(3) 5	3 (5)	5	5
Summit..................	Lobed	Long lobed	Long lobed	Long lobed
Palea:				
Shape....................	Elliptic	Elliptic	Narrow elliptic	Narrow elliptic
No. of nerves.............	2	2	2	2
Anthers:				
Dimorphism..............	Equal	Equal	Equal	Equal
Abortion.................	All normal	All normal	All normal	All normal
No. of beards per sac.......	(4) 10–12 (18)	0–6	0	0–(4)
Awn:				
Persistence..............	Deciduous	Deciduous	Deciduous	Persistent
Geniculation.............	Straight	Straight	Weakly bent	Twice geniculate
Texture..................	Scaberulous	Scaberulous	Scaberulous	Scaberulous
Throat of sheath............	Pubescent	Glabrous	Glabrous	Glabrous
Pollen....................	Normal	Normal	Normal	Normal

* Data for O. hymenoides from JOHNSON and ROGLER, Amer. Jour. Bot.

† Numbers in parentheses represent a few exceptional cases.

The hairs of the lemma also are much longer than in either of those sections. These characters, however, are found in the genus *Stipa*, in which the majority of species have long, narrowly acuminate glumes and some species have pilose lemmas.

The somatic chromosome number of *O. hymenoides*, as reported by STEBBINS and LOVE (16) and confirmed by JOHNSON and ROGLER (10), is 48. The idiogram (fig. 31) is in general similar to that for the other polyploid species of *Oryzopsis*, showing a range in chromosome length of $3-1.2\mu$. On the basis of chromosome number, *O. hymenoides* might be a polyploid from the line in the section *Piptatherum*, with $x = 12$ (or 6) chromosomes. However, in length fully half the chromosomes of *O. hymenoides* exceed the longest chromosomes of either *O. miliacea* or *O. holciformis*.

It was shown by JOHNSON and ROGLER (10) that *O. caduca* Beal is a sterile hybrid between *O. hymenoides* and *Stipa viridula* Trin. Later, JOHNSON (11) pointed out that *O. bloomeri* (Boland.) Ricker is a parallel sterile hybrid between *O. hymenoides* and *Stipa occidentalis* Thurb., and that a number of species of *Stipa* are involved in producing such hybrids with *O. hymenoides*. The ease with which it hybridizes with various species of *Stipa*, taken together with evidence from morphological and cytological characters, brings out the possibility that *O. hymenoides* is an allopolyploid between the line in *Oryzopsis* with a basic number of $x = 12$ (or 6) chromosomes and the line in *Stipa* with a similar basic number. Another possibility is that *O. hymenoides* may be derived from the $x = 12$ (or 6) line of *Stipa* alone without involving any species of *Piptatherum* affinities. This species ranges (fig. 51) throughout the western

half of the United States, where the genus *Stipa* is best developed in North America.

A form of *O. hymenoides* with contracted panicles but similar to the species as generally known in other respects has been collected at Dubois, Idaho. Arthur Cronquist 681 (UIS); 1940, Ray J. Davis (UIS).

A previously undescribed variety of *O. hymenoides* (figs. 68–72; tables 9, 10) is represented by four collections from Colorado and Wyoming (fig. 52).

Oryzopsis hymenoides (Roem. and Schult.) Ricker var. **contracta**, var. nov., a forma typica differt panicula contracta, ramis et pedicellis erectis, pedicellis brevioribus spiculis (cum aristis), villis lemmatis dimidio brevioribus lemmate, arista 2.5-plo longiore quam lemmate.

Culms erect, tufted, 2.5-5 dm. high; culm leaves 3, smooth, narrowly involute, the basal one about 20 cm. long, the upper one 4–10 cm. long; uppermost sheaths dilated; ligule 3–6 mm. long; panicle 6–20 cm. long, narrowly contracted, the branches slender, erect in pairs, the ultimate pedicels erect, shorter than the awned spikelet; glumes about 5.5–6 mm. long, puberulent to glabrous, narrow-ovate, 3–5-nerved, abruptly acuminate; lemma fusiform, turgid, about 3 mm. long, with lobes extending more than 0.5 mm. beyond the joint of the awn, 3- (rarely 5-) nerved, dark brown at maturity, densely pubescent with hairs less than 1.5 mm. long; awn slightly bent, 7–8 mm. long, scaberulous, deciduous; anthers bearded.

Wyoming: Freezeout Hills, Carbon County, Elias Nelson 4850 (type) (UWy); Laramie, Albany County, Elias Nelson 411 (UWy); Leucite Hills, Sweetwater County, Merrill and Wilcox 84 (UWy). Colorado: Laramie River, Elias Nelson 467 (UWy).

The above cited herbarium specimens were used in compiling the data for *O. hymenoides* var. *contracta* in tables 9 and 10. The data for *O. hymenoides* were taken from JOHNSON and ROGLER (10).

V. SPECIES EXCLUDED FROM ORYZOPSIS

1. *Oryzopsis caduca* Beal, Bot. Gaz. 15:111. 1890 = × *Stiporyzopsis caduca* (Beal) Johnson and Rogler (*Oryzopsis hymenoides* × *Stipa viridula*). Amer. Jour. Bot. 30:49–56. 1943.

2. *Oryzopsis bloomeri* (Boland.) Ricker Ex Piper, Contrib. U.S. Natl. Herb. 11:109, 1906; based on *Stipa bloomeri* Boland., Proc. Calif. Acad. Sci. 4:168. 1872, shown by Johnson (11) to be a hybrid between *Oryzopsis hymenoides* and *Stipa occidentalis*.

3. *Oryzopsis webberi* (Thurb.) Benth.; Vasey, Grasses U.S. 23. 1883, based on *Eriocoma webberi* Thurb., in S. Wats., Bot. Calif. 2:283, 1880. Sierra Valley, Calif., Boland. = *Stipa webberi* (Thurb.) comb. nov.

In describing this species, THURBER (in WATSON, 19) "doubtfully referred" it to the genus *Eriocoma*, although Bolander, the collector, had sent it to him with a set of his species of *Stipa* "to which he supposed it to belong." There can be little doubt that Bolander was correct. Its assignment to *Eriocoma*, which was known almost exclusively from the single species now known as *Oryzopsis hymenoides*, was apparently done on the basis of a single character. *Stipa webberi* (figs. 77–80; tables 9, 10) resembles *O. hymenoides* in the long hairs of the lemma and in the deciduousness of the awn, but in the majority of characters it differs from *O. hymenoides* and resembles *Stipa pinetorum* Jones (figs. 73–76; tables 9, 10). It has a long, narrow, non-indurate lemma with a slender callus and long, pointed lobes at the summit. This lemma is quite different from that of *O. hymenoides* but very similar to that of *S. pinetorum*. The latter species also bears long hairs on the lemma. The glumes of *S. webberi* are narrow, as in *S. pinetorum*. The anthers are long and slender, of the *Stipa* type and without beards. The panicle in both species is narrow with appressed branches and frequently has the basal portion included within the somewhat inflated uppermost sheath. This is totally unlike the typical panicle in *O. hymenoides* and in detail does not resemble the more narrow panicle of *O. hymenoides* var. *contracta*. The ligule in *S. webberi* is extremely short, less than 0.5 mm. long (as in *S. pinetorum*), while in *O. hymenoides* it is about 5.0 mm. long.

S. pinetorum and *S. webberi* (fig. 53) occupy the same geographic range, with the former occurring at high altitudes in open pine woods and the latter on the deserts and plains.

In this study, *S. webberi* was found to have 2n = 32 chromosomes (fig. 23). Several counts of n = 16 were also obtained at diakinesis. This number is not easily explained on the basis of other counts in the genus *Oryzopsis*, where the species seem to be either diploids or polyploids of the euploid type. In *Stipa* an aneuploid series is known to exist. In personal correspondence, STEBBINS has reported a count of 2n = 32 chromosomes for *S. pinetorum* also. The idiogram for *S. webberi* (fig. 32) shows chromosomes corresponding in length to those of the stipoid species and the polyploids of *Oryzopsis*.

It is clear that the only character which would retain this species in the genus *Oryzopsis* is the deciduousness of the awn. Its affinity is obviously with *Stipa*.

The following herbarium specimens were used in compiling the data for tables 9 and 10:

Stipa webberi.—Idaho: Owyhee County, reproduced at Pullman, Washington, 1942, Schwendiman (UM). Colorado: Grand Junction, 1900, Stokes (UC).

Nevada: Washoe County, Saarni 104 (UC); Elko County, Holmgren 617 (UWy); Esmeralda County, Duran 3088 (UM). California: Lassen County, Keck and Clausen 3749 (UC).

Stipa pinetorum.—California: Mono County, Sharsmith 4206 (UM).
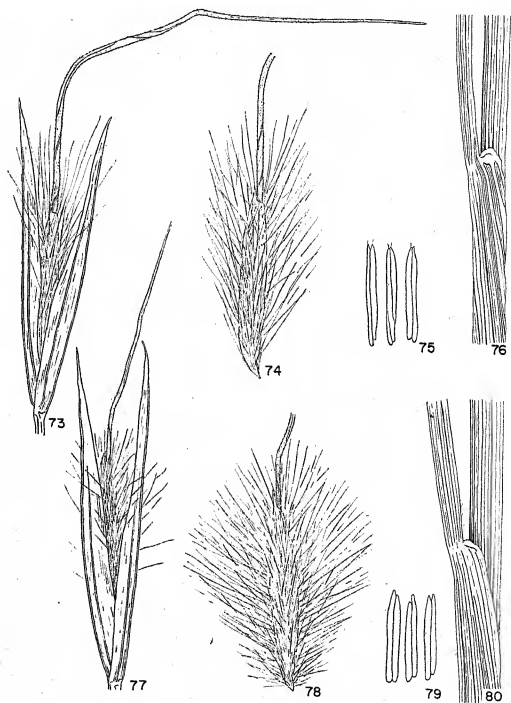
FIGS. 73–80.—Floral and vegetative parts: Figs. 73–76, *Stipa pinetorum;* fig. 73, spikelet; fig. 74, lemma; fig. 75, anther set; fig. 76, throat of sheath. Figs. 77–80, *Stipa webberi;* fig. 77, spikelet; fig. 78, lemma; fig. 79, anther set; fig. 80, throat of sheath. All ×10.

Conclusions

In the subtribe Stipeae the genera *Oryzopsis* and *Stipa* have apparently originated from a common basic stock with x = 6 chromosomes. From this basic stock two diverging lines (fig. 81), each with x = 12 (or 6) chromosomes, seem to have given rise to the genus *Stipa* on the one hand and mainly to the section *Piptatherum* of the genus *Oryzopsis* on the other hand. The absence of a series of intergrading forms indicates that isolation of these two lines is at present fairly complete. However, the polyploid species *O. hymenoides* which constitutes the section *Eriocoma* has the same basic chromosome number and combines some of the more specialized characters from both lines. This suggests that isolation of these lines has been overcome by allopolyploidy in this case.

An early change to a secondary basic number of x = 11 appears to have tentatively isolated a portion of the x = 12 (or 6) line leading to *Stipa*. Some of the less specialized species in this new x = 11 line constitute the major part of the section *Euoryzopsis* in the genus *Oryzopsis*. On morphological characters these species intergrade completely with *Stipa;* but at present, on the basis of x = 11, only diploids are known for *Oryzopsis* and only polyploids for *Stipa*. While the x = 11 line is presumably isolated from the x = 12 (or 6) line in the section *Piptatherum*, recombination between them evidently has occurred through the formation of allopolyploids such as *O. racemosa* and *O. asperifolia*, which combine the chromosome numbers as well as some of the more specialized morphological characters from both lines. Similar recombination between the x = 11 and the x = 12 (or 6) lines in *Stipa* has

probably given rise in part to the modified polyploid series reported for that genus.

In explaining the origin of the secondary basic number of x = 11 in the genus *Stipa*, Avdulov (2) suggests that the number 44 may have occurred after the loss of chromosomes in tetraploid forms with a basic number of x = 12. In the present study the counts of 2n = 22 chromosomes for species of *Oryzopsis* which intergrade with *Stipa* indicate that the basic number of x = 11 might more probably have originated in a species with 2n = 24 chromosomes. However, a consideration of morphological characters together with chromosome numbers presents a more likely explanation for the origin of the basic number, x = 11.

As previously pointed out, in the section *Euoryzopsis* the four species *O. pungens*, *O. canadensis*, *O. exigua*, and *O. kingii* form a coherent group possessing stipoid characters, with *O. micrantha* somewhat detached from these owing to its possession of some *Piptatherum* characters. Counts of 2n = 22 chromosomes occur in the more stipoid group as well as in *O. micrantha*. A change from 2n = 24 to 2n = 22 in the x = 12 line leading to *Stipa* would not account for the *Piptatherum* characters of *O. micrantha*, nor would such a change in the *Piptatherum* line account for the *Stipa* characters of the other four species. It has been suggested, however, that the primary basic number in *Stipa* is x = 6 rather than x = 12, and this may apply as well to *Oryzopsis*. A single change from 2n = 12 to 2n = 10 in *Stipa*, with subsequent formation of allopolyploids, could account for both the origin of the basic number x = 11 and the combination of characters exhibited by *O. micrantha*.
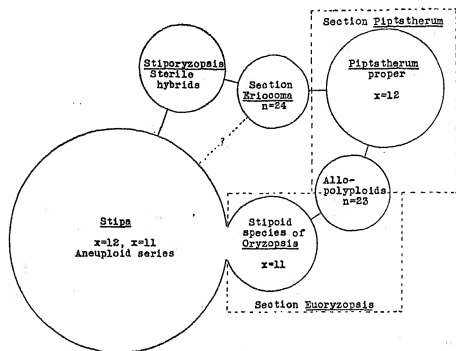
81
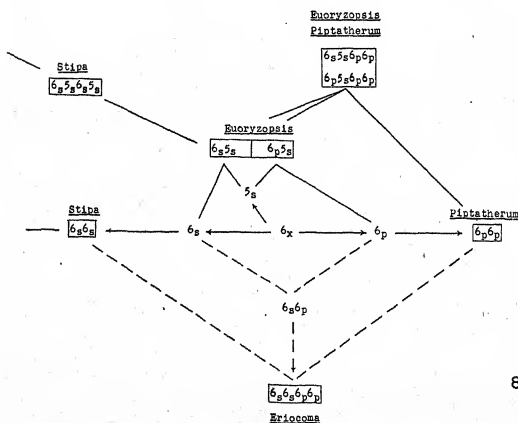
82

Figs. 81, 82.—Fig. 81, diagram of natural groups in *Oryzopsis* showing probable interrelationships and general affinities to *Stipa*. Fig. 82, suggested pattern of differentiation of *Stipa* and *Piptatherum* genomes from common stock and their reduplication and recombination to give secondary basic and higher chromosome numbers associated with complexes of morphological characters in *Oryzopsis* and with generic characters of *Stipa*.

Assuming that the primary basic number for both *Stipa* and *Oryzopsis* is $x = 6$, the primitive genome may be designated as 6_x (fig. 82). By specialization in different directions, the *Stipa* genome 6_s and the *Piptatherum* genome 6_p may have been derived from the primitive one. Doubling of the chromosomes in each to give 6_s6_s and 6_p6_p would explain the counts of $2n = 24$ cited for *Stipa sibirica* and for *O. miliacea*, *O. virescens*, and *O. holciformis* of the section *Piptatherum*. A single change in chromosome number in the 6_s genome could have produced a 5_s genome which may have had only a temporary existence or may have formed a third basic number in *Stipa*. The formation of one or more allopolyploids between the 6_s and 5_s genomes would account for the stipoid species *O. pungens* and *O. kingii* with $2n = 22$ chromosomes now classed in *Oryzopsis*. Allopolyploidy between the 6_p and 5_s genomes would account for *O. micrantha* with $2n = 22$ chromosomes and with a combination of *Stipa* and *Piptatherum* characters. Doubling of the chromosome number in 6_s5_s species of the $x = 11$ line could give rise to $6_s6_s5_s5_s$ polyploids, represented by $2n = 44$ counts in *Stipa*. The higher allopolyploids, *O. racemosa* and *O. asperifolia*, may have been formed by combination of a 6_s5_s or 6_p5_s genome with a 6_p6_p genome to give $6_s5_s6_p6_p$ or $6_p5_s6_p6_p$. The origin of *O. hymenoides* may have been by the formation of a 6_s6_p combination, with subsequent doubling, or by direct formation of a $6_s6_s6_p6_p$ combination from the 6_s6_s and 6_p6_p lines. In the genus *Stipa* various combinations of 6_s, 6_s6_s, 6_s5_s, and possibly 5_s genomes may account in part for its modified polyploid series. Certain counts in *Stipa* suggest that a third basic number of $x = 5$ may exist. For example, a simple backcross of a 6_s5_s genome to a 5_s genome and subsequent doubling would explain the count of $2n = 32$ found in *S. webberi*.

It appears, therefore, that in the genus *Oryzopsis* the taxonomist has to contend not only with divergence of lines from a less specialized type but also with the convergence of these lines in allopolyploids which combine the characters of more highly specialized types. While all the hybrids between *O. hymenoides* and *Stipa* examined have been found to be sterile, the opportunity is nevertheless present from year to year for such perennial plants to form additional allopolyploids by doubling of their chromosome number and thus continue the process of reticulation between otherwise independent lines.

Such divergence and later convergence in the phylogenetic lines of a portion of the subtribe Stipeae have resulted in a large coherent group of species represented by the genus *Stipa* (fig. 81) and in a number of smaller peripheral groups which constitute the genus *Oryzopsis*. The groups in the latter genus are small aggregates of species together with the polyploids formed between these aggregates. In addition, hybrids are formed between one of these groups and the genus *Stipa*. Thus, an attempt to reduce the genera so as to include only species which are lineally related would still leave a few species which are intermediate between such genera and which do not among themselves constitute a homogeneous group.

The question of where to draw the boundary between the genus *Stipa* and the group of stipoid species of *Oryzopsis* is one which is frequently met among less specialized members of related genera. As judged from the chromosome counts which have been made, the line as drawn at present places the diploid

species with $2n = 22$ chromosomes in *Oryzopsis* and the polyploids in *Stipa*. On the basis of morphological characters the separation is not clear, but to draw the line elsewhere would still not effect a distinct separation.

With the removal of *Stipa webberi* and the hybrids previously known as *Oryzopsis caduca* and *O. bloomeri* from the genus *Oryzopsis*, the latter is then left with a number of species whose interrelationships among themselves and whose affinities to *Stipa* can be interpreted.

Summary

1. The Old World species *Oryzopsis miliacea*, *O. virescens*, *O. paradoxa*, *O. coerulescens*, and *O. holciformis*—which constitute most of the section *Piptatherum*—form a coherent group marked by specialization in characters which clearly distinguish them from the genus *Stipa*. On these characters *O. virescens* is most representative of the genus *Oryzopsis*, while *O. holciformis* shows the greatest specialization or departure from the generic characters of *Oryzopsis* and still greater divergence from the generic characters of *Stipa*. *O. miliacea* shows evidence of reduction from a specialized condition. *O. holciformis* and *O. miliacea* were found to have $2n = 24$ chromosomes. Their idiograms are similar and both differ notably from the idiograms of other species of *Oryzopsis* studied. Previous counts of $2n = 24$ have been reported for *O. miliacea* and *O. virescens*. It is concluded that the basic number for the section *Piptatherum* is $x = 12$ (or 6). This has been previously suggested as the basic number for the genus *Oryzopsis* and as the primary basic number for the genus *Stipa*.

2. The North American species *O. micrantha*, *O. pungens*, *O. exigua*, *O. canadensis*, and *O. kingii*—which con-
stitute most of the section *Euoryzopsis*—form a group marked by specialization in characters which merge with the genus *Stipa*. *O. micrantha* is somewhat detached from the other species by its resemblance to the section *Piptatherum* on some characters, while the highly specialized *O. kingii* departs farthest from the generic characters of *Oryzopsis* and intergrades completely with *Stipa*. Chromosome counts of $2n = 22$ were obtained for *O. micrantha*, *O. pungens*, and *O. kingii*. Their idiograms are similar, and all have longer chromosomes than in the Old World species. It is concluded that the basic chromosome number for the section *Euoryzopsis* is $x = 11$. A secondary basic number of $x = 11$ has been reported previously for *Stipa*.   *O.*

3. The North American species *racemosa* and *O. asperifolia* combine morphological characters from the two lines in *Oryzopsis* with $x = 12$ (or 6) and $x = 11$ chromosomes. In size of plants and floral parts, they are larger than the species of either line. Both were found to have $2n = 46$ chromosomes. The idiograms show that in range of chromosome lengths *O. racemosa* includes most of the range for the $x = 12$ (or 6) line plus most of the range for the $x = 11$ line. It is concluded that they are allopolyploids between those two lines. *O. racemosa* is referred to the section *Piptatherum*. *O. asperifolia* is the type of the section *Euoryzopsis*. Another species, *O. hendersoni*, may represent another such allopolyploid, as judged from morphological characters. It is referred to *Euoryzopsis*.

4. The North American species *O. hymenoides*, which is the only one in the section *Eriocoma*, resembles the genus *Stipa* in some features and *O. virescens* of the section *Piptatherum* in other features. A count of $2n = 48$ chromosomes has been previously reported for *O.*

hymenoides. Its idiogram shows a range in chromosome lengths which greatly exceeds that for the $x = 12$ (or 6) line in *Oryzopsis*. It may be an allopolyploid between that line and the line in *Stipa* with a similar basic number or possibly a polyploid from the *Stipa* line alone. It hybridizes naturally with a number of species of that genus to produce sterile hybrids.

5. A variety of *O. hymenoides* with a contracted panicle is described.

6. *O. webberi* is transferred to *Stipa* on the basis of its close similarity to *S. pinetorum*. Both species have $2n = 32$ chromosomes.

7. *O. bloomeri* and *O. caduca* are excluded from *Oryzopsis* on the basis of proof published elsewhere showing that they are sterile hybrids between *O. hymenoides* on the one hand and *S. occidentalis* and *S. viridula*, respectively, on the other hand.

8. It is concluded that the section *Piptatherum* and the genus *Stipa* in part represent divergent lines of specialization on a common primary basic number of $x = 6$ chromosomes and that these lines may have reticulated to form the polyploid section *Eriocoma*. The section *Euoryzopsis* represents mainly the diploid species of a line that has tentatively become isolated from the $x = 12$ (or 6) lines by a change to a secondary basic number of $x = 11$. The latter line in turn has reticulated with the *Piptatherum* line to form allopolyploid species of *Oryzopsis* and with the *Stipa* line to contribute to the modified polyploid series reported for that genus.

9. It is suggested that the secondary basic number of $x = 11$ probably originated through a change from $2n = 12$ to $2n = 10$ within the *Stipa* line, with subsequent allopolyploidy between $2n = 10$ individuals and the original $2n = 12$ lines in both *Stipa* and *Piptatherum*.

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INHERITANCE OF THE MAIN ANTHOCYANIN PIGMENTATION AND OF SOME OF ITS PATTERNS IN FLOWERS OF NEMESIA STRUMOSA[1]

HERBERT PARKES RILEY

The writer has shown that a number of genes are involved in the determination of flower color in *Nemesia strumosa* Benth., a South African member of the Scrophulariaceae (23, 24, 25). The white-flowered type can be regarded as the standard. It has a two-lipped corolla whose limb is white (or perhaps ivory, comparable with LAWRENCE's [14] ivory-colored dahlia). The lower part of the short tube is orange and frequently contains deep purple spots which vary greatly in number and size and which frequently are so dark-hued as to appear black. These purple spots may not be present, but, if they are, they are accompanied by a deep purple spot on the upper lip just above the stamens.

One of the main variants is the orange type, which differs from the white-flowered form by the presence of a deep orange-yellow sap pigment in the epidermal cells of the inner part of the corolla

limb. Orange is a simple dominant to white and is determined by gene *O*. The orange type can be modified by other genes, of which two have been analyzed. The recessive gene for pale-upper, *p*, causes the upper lip of orange-colored flowers to be considerably lighter in hue, while the lower lip is unaffected. The recessive gene for buff, *bu*, modifies the lower lip to Buff Yellow[2] and the upper lip to Sulphur Yellow with areas of Buff Yellow. Gene *bu* is linked with the self-sterility alleles, with about 5% crossing over, and is epistatic to gene *p*.

Three recessive genes are responsible for the presence of yellow pigmentation in *oo* plants. Gene *gl* (glass-green) produces a color in the limb which is usually close to Sea-foam Yellow or Pale Glass-Green, but *glgl* are sometimes so pale as to be difficult to distinguish from white. A deeper yellow color is produced by the recessive gene, *y* (yellow). Yellow plants are not so deep as orange and may vary from slightly darker than glass-green at the one extreme to almost buff at the

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[1] This paper is one of a series on the genetics of the genus *Nemesia*. Some of the data were collected at Princeton University and as a National Research Council fellow in the Biological Sciences at the Bussey Institution of Harvard University. Later families were grown at the University of Washington (Seattle) and at the University of Kentucky.

[2] Throughout this paper, the names of colors which are capitalized are those listed as such in RIDGWAY (21).

other. Because of this variation, and because of the resemblance to buff, it has presented some difficulty. The yellow-spot gene (ys) produces a number of dots or streaks radiating from a small yellow area on the lower lip, near the throat.

As in the case of many other plants, one of the common flower-color types in *N. strumosa* is the "colored" type that contains anthocyanin pigment in the cells of the corolla and which appears as some shade of red or purple. That this type is due to a single dominant gene, *C*, has been previously mentioned (23).

Anthocyanin pigmentation was one of the first characters studied in genetics, being one of the characters in MENDEL'S (18) original experiments. Experiment 3 of his paper deals with the color of the seed coat, flowers, and stems of the pea. In plants with the dominant gene, the seed coats are gray, gray-brown, or leather-brown, and opaque; the standards of the flowers are violet and the wings are purple; while the stems in the axils of the leaves are of a reddish tint. In the homozygous recessive, the seed coats are transparent, the flowers are white, and there is no red color in the stems. In some experiments with plants other than the pea, MENDEL crossed the white-flowered *Phaseolus nanus* with the purple-red-flowered *Ph. multiflorus*. The first species also had white seeds, while the latter had red seeds with black flecks and splashes. This color difference was not the result of a single gene, and MENDEL considered that the result "might probably be explained by the law governing *Pisum* if we might assume that the colour of the flowers and seeds of *Ph. multiflorus* is a combination of two or more entirely independent colours, which individually act like any other constant character in the plant." Anthocyanin

pigmentation was also reported in a number of other plants in 1900 and 1901. For example, the dominance of colored to noncolored was demonstrated in *Agrostemma, Atropa, Belladonna, Clarkia pulchella, Hyoscyamus* hybrids, *Lychnis, Papaver somniferum, Polemonium coeruleum, Trifolium pratense,* and *Veronica longifolia* by DE VRIES (36); in *Anemone* hybrids by HILDEBRAND (9); in *Epilobium angustifolium* and in *Matthiola* hybrids by CORRENS (5, 6); and in beans by VON TSCHERMAK (34). HILDEBRAND (9), however, found that a cross between a light blue *Anemone angulosa* and a dark blue *A. hepatica* produced an intermediate color in the F_1, while DE VRIES (36) found that in *Coreopsis tinctoria* yellow was dominant to brown and postulated that the yellow-flowered type possessed a gene that inhibits anthocyanin production.

It has been found frequently that the formation of anthocyanin pigment depends upon the simultaneous presence of more than one gene, and that these act as complementary genes. Such a situation has been demonstrated in a number of plants, including *Antirrhinum majus* (3, 4), *Campanula medium* (13), *Cattleya, Cypripedium,* and *Dendrobium* (10), *Dolichos lablab* (8), *Ipomoea purpurea* (1), *Lathyrus odoratus* (2), *Linum usitatissimum* (33), *Lychnis* (32), *Matthiola incana* (35, 28), *Mirabilis jalapa* (17), *Pharbitis nil* (11), *Phaseolus vulgaris* (31), *Phlox drummondii* (12), and *Solanum tuberosum* (26). In a number of instances, the two complementary genes have been designated *C* and *R*. Complementary genes for anthocyanin pigment have been found in so many different plants that SANSOME and PHILP (27) conclude, "It is, however, significant that the *CR* type of inheritance of colour is widespread among plants and is prob-

ably connected with anthocyanin pigments in almost every case."

In some plants the situations may be somewhat different, as in LAWRENCE'S (14) study of *Dahlia variabilis*, where gene *A* produces a relatively pale color while *B* produces a deeper color; both genes appear to be cumulative in their action.

In many plants both anthocyanin and yellow sap pigments may be present. In some of these cases it has been demonstrated that the anthocyanin color is red if a yellow pigment is present but magenta if the background is ivory rather than yellow. In the snapdragon, for example, gene *Y* produces yellow color owing to the presence of a glucoside of luteolin in the corolla lips, while gene *I* produces an ivory color owing to a glucoside of apigenin. In yellow-flowered plants, both glucosides are present. Apparently gene *I* acts only in the presence of *Y*, for *yyI-* and *yyii* are white and have no flavone pigment (37, 38, 39, 19, 20, 29). When anthocyanin pigment is present in *Y-ii* (yellow) plants, its color is crimson, but if it is present in *Y-I-* (ivory) plants, it is magenta. On the other hand, in Indian cotton, LEAKE (15) failed to distinguish between anthocyanin plus yellow and anthocyanin plus white. He found that the flowers of *Y-RR* plants were red, those of *Y-Rr* were red on yellow, while those of *yyRR* and *yyRr* were red on white.

LAWRENCE'S (14) observations on *Dahlia variabilis* are interesting, as the background may be yellow, cream, ivory, or white, because two genes for anthocyanin are present, because each appears to act cumulatively, and because each is tetrasomic since the species is an octoploid. The background is determined principally by genes *Y* and *I*. Gene *Y* produces yellow pigment and is complete-

ly epistatic to *I*, so that all *YI* and *Yi* combinations are yellow; gene *Y* is tetrasomic. Gene *I*, which is disomic, produces an ivory flower in the absence of *Y*. All *yi* plants are white. In addition, genes which inhibit the *Y* gene may be present and produce a cream type. The *B* gene produces a deeper anthocyanin pigment than does *A*, but both genes tend to be on the magenta side with ivory and on the orange-scarlet side with yellow. Thus, *A* in the simplex condition is pale magenta with ivory and apricot with yellow, while *B* simplex is, respectively, purple and scarlet. Additional *A* and *B* genes (or both) deepen the color, but in all cases the colors remain within the ranges. Unanalyzed, modifying genes also vary the shade and may also affect the intensity of the color. For example, in some families the magenta class (*Aby*) is "easy to score and very distinct"; but when the Union Jack variety is one of the parents, there is a considerable deficiency of magentas but an excess of the deeper rosy-purple. Similarly, in apricot flowers, if there is a mutation of the *Y* gene to *y* so as to produce ivory sectorial chimaeras, the magenta color of the sector is considerably deeper than the apricot regions next to it, suggesting that modifying genes have a different effect on *AI* from that produced on *AY* regions. Another factor that influences the color of anthocyanin types is the intensity of the yellow background color. If it is a good yellow, certain genotypes may appear scarlet as expected; if, however, yellow-inhibiting genes are present and the background is cream, the same genotypes (exclusive of the inhibitor) may appear purple and resemble a plant with the same *A* and *B* gene combination but with an ivory background. Since certain anthocyanin-gene combinations may give the same result with *YI* and with

$Y i$ plus a Y inhibitor, the scoring of the background in "colored" plants is often subject to considerable error.

Observations

The materials used for this study were the same as those described previously

ground is white (*oo*). Owing probably to the presence of numerous modifying genes, there is some overlapping of phenotypes, and an attempt to determine the background by examining the color of flowers with anthocyanin pigmentation is subject to considerable error.
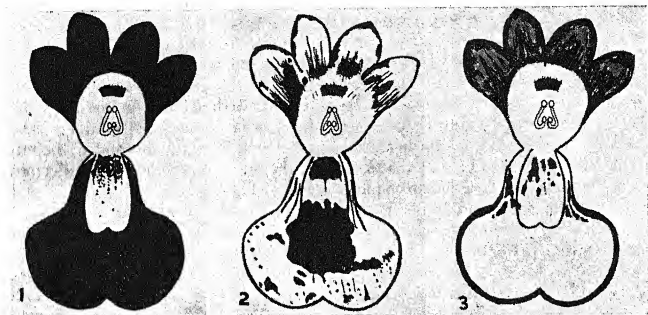
FIGS. 1–3.—Diagrams of flowers of *Nemesia strumosa*. Each flower is cut along both sides of tube and then flattened out (causing some distortion of the tube but little of the lips). Fig. 1, self-colored purple or red type; *C Sp Gr Ro*. Fig. 2, splotched; *C sp Gr Ro*. Fig. 3, red-outline; *C Sp Gr ro*. In each flower, blackened areas show distribution of anthocyanin (red or purple) pigmentation, except that black areas and dots in rear of lower part of tube and black "eyebrow" over stamens represent very deep purple pigmentation caused by another gene. For comparison with standard type, see 25.

(25). In fact, many of the families reported in that paper were the same as those involved in this study.

COLORED (C)

The white-flowered type which has been taken as the standard in this work has been described previously. In the colored type the inner part of the corolla limb is a shade of purple or red instead of white, but otherwise the white and colored types are similar with respect to the inner part of the corolla (fig. 1). As in some other plants, the tendency is toward the red shades if the background is orange owing to the dominant gene *O*, but magenta or purplish if the back-

Probably the most frequent colors of the anthocyanin in combination with white are Pomegranate Purple or Amaranth Purple, while the most frequent anthocyanin-orange types are Carmine or Oxblood Red. However, other colors frequently found on a white background are Tyrian Rose and Rose Color and on an orange background are Bordeaux and Garnet Brown.

In Pomegranate Purple plants, the cells of the inner epidermis of the limb of the corolla contain a purplish red pigment in solution in the cell sap. When ammonia is added, most of the cells turn olive brown, and many turn olive green, while a few become bright green. When

hydrochloric acid is added, the colored cells turn bright red. Further chemical tests have not been made, but it is probable that this pigment is the same as or is closely related to the cyanidin 3-bioside reported for pink and crimson *Nemesia* hybrids by Scott-Moncrieff (30). In the throat most of the cells are orange, but there are numerous areas of cells which contain a very deep purple pigment, and an occasional "colored" cell is bright red instead of deep purple. The throat is similar to that in white-flowered plants, and the deep purple areas are probably the result of another gene, which will be reported in a subsequent paper. In the area where the lip connects with the tube, in "colored" plants on a white background most of the cells are purple, owing to the anthocyanin gene, but some are orange; in the tube just back of this region there are both purple and orange cells, with the latter becoming more frequent the farther they are from the lip. These purple cells are not to be confused with the deep purple, almost black, cells of the "black" spots of the tube. In the throat region all the long hairs are yellow, even in purple ("colored" as distinguished from "black") areas.

When a purple-flowered plant is placed in ammonia, dark green spots soon appear in the purple regions, and the white (or ivory) regions at the sides of the throat turn yellow. When the epidermis is stripped off, the white regions beneath turn yellow. The lips continue to turn green and finally become very dark. In sulphur dioxide the lips fade to Rose Color but do not seem to fade out entirely. In hydrochloric-acid vapor, the lips turn close to Nopal Red.

That colored is dominant to noncolored is observed from a number of crosses in both the old and new material. In two families, crosses between two colored plants yielded 140 colored and no noncolored. In twenty-two families from crosses between two noncolored, there were no colored plants and 1146 noncolored. Crosses between a noncolored and a colored that must have been homozygous were made in three cases and produced 190 colored and no noncolored offspring. Eight families resulted from a cross between two heterozygotes. The total was 403 colored and 172 noncolored, and the results in the individual families have been listed in table 1. When these eight families are totaled, the ratio of the deviation to the standard error is 2.72, which indicates a probability of occurrence of only about 0.69%, although in no individual family is the deviation significant. In each of the eight families there is a deficiency of colored plants which is not large enough to be significant in any one family but which is significant when the eight families are combined. The reason for this slight deficiency of colored plants cannot readily be explained. Whenever disturbed ratios occur in plants which are self-sterile, linkage with the self-sterility alleles must be considered as a possible explanation. While such an explanation will fit certain of the families in table 1 it will not fit others, and if all the families in tables 1 and 2 are diagrammed, it becomes apparent that linkage with the s genes is not the explanation. For example, family 3209 arose from the cross 3112(11) $\times$ 3211(4); it has been shown (22) that the self-sterility alleles of the parents are s^2s^4 and s^1s^2. The female parent was white, so that, if there were linkage, its genetic constitution would be s^2c/s^4c. Plant 3209(3) was purple and s^1s^2, so it could only be s^1C/s^2c, since its s^2 gamete must have come from the noncolored female parent. When this is crossed as a

male on to 3211(4), a Cc plant with the constitution s^2s^3, there is a decided deficiency of colored plants in the offspring (family 3374). On the basis of linkage between c and s, however, an excess of coloreds would be expected, because the s^2

served ratio of 26:29 shows no significant deficiency. Other similar examples might be cited to show that the consistent slight deficiency of C plants in the eight F_2 families is not due to linkage of C with the self-sterility alleles.

TABLE 1

RATIO OF COLORED TO NONCOLORED IN EIGHT FAMILIES, OF WHICH BOTH PARENTS WERE HETEROZYGOUS FOR C

FAMILY	PARENTS	OBSERVED		EXPECTED		Dev. S.E.
		C	c	C	c	
3004.......	2901(7) × (13)	70	25	71.25	23.75	0.30
3012.......	2901(13) × (10)	61	31	69.0	23.0	1.93
3211.......	3111(4) × (5)	31	16	35.25	11.75	1.43
3214.......	3111(5) × (4)	17	7	18.0	6.0	0.47
3226.......	3103(5) × (3)	44	22	49.5	16.5	1.56
3366.......	3206(2) × (1)	52	20	54.0	18.0	0.56
3372.......	3209(3) × 3226(7)	60	21	60.75	20.25	0.19
3374.......	3111(4) × 3209(3)	71	30	75.75	25.25	1.09
Total....	....	406	172	433.50	144.50	2.64

TABLE 2

RATIO OF COLORED TO NONCOLORED IN EIGHT TESTCROSS FAMILIES IN WHICH FEMALE IS RECESSIVE AND MALE HETEROZYGOUS. IN THE FIRST THREE FAMILIES, BACKGROUND IS ORANGE; IN THE OTHER FIVE IT IS WHITE

FAMILY	PARENTS	SELF-STERILITY ALLELES	OBSERVED		EXPECTED		Dev. S.E.
			C	c	C	c	
3103........	3001(6) × (22)	$s^x s^y × s^y s^z$	35	44	39.5	39.5	1.01
3225........	3103(1) × (5)	$s^y s^z × s^x s^z$	14	16	15.0	15.0	0.36
3361........	3103(52) × (4)	$s^x s^y × s^y s^z$	15	7	11.0	11.0	1.70
3206........	3112(10) × 3111(4)	$s^2 s^3 × s^1 s^2$	6	3	4.5	4.5	1.00
3209........	3112(11) × 3111(4)	$s^2 s^4 × s^1 s^2$	5	7	6.0	6.0	0.58
3217........	3111(3) × (4)	$s^1 s^3 × s^1 s^2$	28	12	20.0	20.0	2.50
3367........	3209(1) × (3)	$s^1 s^4 × s^1 s^2$	26	29	27.5	27.5	0.40
3406........	3206(6) × (1)	$s^1 s^3 × s^1 s^2$	16	6	11.0	11.0	2.13
Total....	....	....	145	124	134.5	134.5	1.28

gamete would be eliminated from the male parent, and this is coupled with the c gene. Likewise, family 3367 arose from the cross 3209(1) × 3209(3) and should show a significant deficiency of C plants when compared with a 1:1 ratio. The ob-

In thirty-two testcross families the combined ratio was 687 colored:648 noncolored, and only two families deviated significantly from expectation. In eight of the families the male was heterozygous and there was a common self-sterili-

ty allele, and six agreed well (table 2) with expectation on the basis of no linkage. Four showed a deficiency of C and four of c plants.

The dominant gene for the main anthocyanin pigmentation will be designated C. This pigmentation has been referred to as the *main* anthocyanin pigmentation, because several other cases of anthocyanin pigmentation due apparently to entirely different genes have been found and will be reported in a subsequent paper.

It has been shown that, in a number of plants, anthocyanin flower color is the result of two complementary genes, usually designated C and R. Either this is not the case in *N. strumosa* or the original plants were all homozygous for one of the dominant color genes. At any rate, there is no evidence for complementary genes, for no families from two colored plants segregated into ratios that approached 9:7, and there were no cases where two noncolored plants produced colored when crossed.

It has been stated that it is difficult in some families to determine from the color of the anthocyanin pigment whether the background is orange or white. In a few families which perhaps lack certain modifying genes this distinction can be made easily. For example, in family 3010, from the cross $CcOo \times CCOo$, there are 73 red :28 purple. The red plants are Ox-blood Red, Burnt Lake, Bordeaux, Scarlet Red, and Coral Pink, and the purple plants are Rose Color. This family is one of the few easy ones to score. The interaction of the anthocyanin pigmentation with such types as buff, pale-upper, yellow, glass-green, or yellow-spot has not been tested.

There is no evidence for linkage between the C and O genes and two families appear to indicate that these genes are

not linked. Family 3004 came from the cross $CcOo \times Ccoo$ and would be expected to segregate into a ratio of 6 colored : 1 orange : 1 white if there is no linkage. The observed ratio is 70 : 10 : 15, which does not deviate significantly from the expected. The parents of family 3006 were $ccoo$ and $CcOo$. This should segregate into 2 colored : 1 orange : 1 white, and the observed ratio of 54 : 22 : 19 agrees with the assumption of no linkage.

It has not been possible in C plants with an orange background to determine whether they are also Bu or bu. Therefore, to determine whether there is any linkage between C and bu, the ratio of Bu to bu in noncolored plants must be examined; it must not be 3 : 1 or 1 : 1. For example, in the testcross $CcBubu \times ccbubu$, a ratio of 2 colored : 1 orange : 1 buff is to be expected if there is no linkage, while the ratio will be 1 colored : 1 buff if linkage is complete and is in the coupling phase. If there is some crossing over, half the offspring will be colored and of the remainder there will be some orange (nonbuff), but the percentage of orange among the noncolored will not equal 50%. Similarly, if the original cross is in the repulsion phase, the ratio will be 1 colored : 1 orange (nonbuff) if linkage is complete; if there is some crossing over, some buff plants will be present, but they will not equal 50% of the noncolored. If bu and C are completely linked and if one parent is homozygous for both bu and c while the other is heterozygous for both and has the two dominant genes on one chromosome and the two recessives on the other, the noncolored offspring will all be buff. The same result will be obtained if bu is not linked with C, but if it is completely linked with the self-sterility alleles and if the female parent is homozygous for both bu and c

while the male is heterozygous for both and the *Bu* gene in the male is on the same chromosome as the common self-sterility allele. Thus the cross *cbu/cbu* × *CBu/cbu* will give the same ratio of *Bu* to *bu* in *cc* plants as will the cross *cc s¹bu/ s²bu* × *Cc s¹Bu/s³bu*. Similarly, the cross *cbu/cbu* × *Cbu/cBu* will yield the same results as the cross *cc s¹bu/s²bu* × *Cc s¹bu/s³Bu*. In such cases it is impossible to determine from the *Bu:bu* ratio alone whether *bu* is linked with *C* or with *s*. On the other hand, in the reciprocal crosses there is no evidence of linkage if *bu* is linked with *s*, but the ratios will be the same if *bu* and *C* are linked no matter in which direction the cross is made. Although several families indicate nothing more than that *bu* is linked with either *C* or *s*, several others indicate that the linkage is with the self-sterility alleles—as has been suggested previously (24, 25). In family 3362 the ratio is disturbed, although there is no *C* gene in either parent, so that it could not be due to linkage with *C*. Family 3004 came from the cross *CcBubuOo* × *Ccbubuoo* and segregated into 70 colored : 6 orange (nonbuff) : 4 (orange) buff : 15 white. The ratio of 1 nonbuff : 1 buff is expected if *bu* is linked with *s*, since the male is recessive, but it would not be expected if *bu* were linked with *C*. There is, therefore, no evidence that *C* is linked with *bu*.

Since genes *P* and *p* cannot be differentiated in *C* plants, any linkage between *p* and *C* must be determined from the *P:p* ratio in the *cO* plants of families which are segregating for *C*. If *p* and *C* are linked, the ratio of *P* to *p* should not be 1:1 or 3:1 in backcross or F₂ families in which some of the plants are *C*. In table 1 of a previous paper (25), eight families segregating for *p* are listed. All but family 3362 are also segregating for *C*. Of the seven families which segregate

for *C*, only one deviates significantly from a 1:1 or 3:1 ratio, and this is a family with only ten *cc* plants. The evidence from these families seems to indicate clearly that *p* is not linked with *C*.

Just as possible linkage between *C* and *bu* or *p* must be tested from the *Bu : bu* and *P : p* ratios in *c* plants, any linkage between *C* and genes *y, gl,* or *ys* must be determined solely from the ratios of these yellow genes and their dominant alleles in the *c* plants of families segregating for *C*. There is only one family that offers any evidence in regard to possible linkage between *C* and *y*. Family 3012 arose from a cross between two *CcYy* plants. As the ratio of white : yellow does not deviate significantly ($\chi^2 = 0.52$; df = 1; P = 30–50%), there is no reason to assume that these genes are linked. Six families have at least one parent heterozygous for both *C* and *gl* and might therefore be used to test possible linkage between these genes. Five families (table 3) agree with the assumption of no linkage, and the sixth does not deviate too badly, although significantly. Three families suggest no linkage between *C* and *ys*, while the fourth (table 3) deviates significantly. In view of the difficulty of scoring *gl* and *ys*, however, it cannot be said that there is any evidence for linkage between *C* and either of these genes.

SPLOTCHED (SP) AND GRANULAR (GR)

Splotched and granular are both modifications of colored and act only on *C* plants. In the earlier material they were found only in the line of plants which had an orange background, except that they were possibly present in families 3005 and 3013, two of the F₁ families that were not used to produce further generations. They have not been tested in the new material. Families 3005 and 3013 were very complicated, segregating for a

large number of genes, and it is possible that some phenotypes that resembled those produced by the splotched and granular genes were actually the result of other genes. The patterns in these families were very difficult to score.

In general, it is not an easy matter to score and to analyze the patterns which appear on C plants. The splotched and granular types are fairly clear, but some of the others have been impossible to study. This is especially true when several pattern genes are present in the same plant. In the splotched type, the anthocyanin pigmentation is not spread evenly over the entire limb of the corolla but is dispersed in a number of lines and splotches, much as in figure 2. As might be expected with a pattern of this sort, there is considerable variation in its details in different plants, and even in different flowers on the same plant. While this is true, the splotched type can usually be recognized rather readily, provided that too many modifying genes are not present. The granular type is more like the normal colored type, in that the color is much more evenly and regularly dis-

tributed than it is in splotched plants. There are, however, numerous small white dots that appear over the entire colored area.

Splotched is apparently recessive to self color, but the data in support of this are not numerous. Family 3009 arose from a nonsplotched colored and a noncolored which presumably was heterozygous for splotched. It segregated into 8 colored nonsplotched : 2 colored splotched : 9 noncolored. Family 3004

TABLE 3

RATIOS OF NONGLASS-GREEN: GLASS-GREEN AND NONYELLOW-SPOT: YELLOW-SPOT IN FAMILIES ALSO SEGREGATING FOR C. IN FIRST SIX FAMILIES, RATIO OF DEVIATION TO STANDARD ERROR REPRESENTS $Gl: gl;$ IN THE LAST FOUR IT STANDS FOR $Ys: ys$

Family	Genotypes of parents	Colored	Orange	White	Glass-green	Yellow-spot	Dev. S.E. (if no linkage)
3004	$Cc\ Glgl \times Cc\ Glgl$	70	10	13	2	....	1.04
3006	$cc\ glgl \times Cc\ Glgl$	53	22	10	9	....	0.23
3111	$Cc\ Glgl \times cc\ Glgl$	16	....	10	4	....	0.31
3215	$Cc\ Glgl \times cc\ Glgl$	18	....	17	5	....	0.25
3374	$Cc\ Glgl \times Cc\ Glgl$	71	....	26	4	....	1.48
3375	$Cc\ Glgl \times cc\ Glgl$	29	....	26	2	....	2.18
3209	$cc\ Ysys \times Cc\ Ysys$	5	....	6	....	1	0.66
3211	$Cc\ Ysys \times Cc\ Ysys$	31	....	13	....	3	0.58
3213	$Cc\ Ysys \times cc\ Ysys$	12	....	14	....	2	1.16
3216	$Cc\ Ysys \times cc\ Ysys$	9	....	2	....	7	3.65

arose from a cross between a colored nonsplotched plant which had a red outline and a colored nonsplotched. It segregated into 20 colored orange nonsplotched : 2 colored orange splotched : 48 colored nonorange : 10 orange : 15 white. The smaller number of splotched in both families is a good indication of the recessive nature of the gene, a supposition which is corroborated by families 3001 and 3103 (table 4). The symbol for the splotched gene shall be sp. Since Sp and sp cannot be distinguished in c plants, the c gene is epistatic to the splotched gene. Family 3004 indicates that in C plants o is also epistatic to Sp and sp.

This, however, needs further corroboration.

Granular also appears to be recessive to self color. Family 3226 arose from two colored, nongranular plants which were apparently heterozygous for both C and the granular gene. This family segregated into 31 colored nongranular : 13 colored granular : 22 noncolored. In family 3001, of which the parents were a noncolored female apparently heterozygous for granular and splotched and a male heterozygous for colored, granular, and splotched, there were 8 self-colored, 1 splotched, and 2 granular. Family 3013 segregated into 6 colored orange : 8 splotched : 3 granular : 23 colored on a white background. The fact that granular plants form such a small percentage of the individuals in these three families suggests that the granular type is the result of a recessive gene. One family (3223) gives a peculiar ratio to appears to contradict this (table 4) and must remain unexplained. Six testcross families totaled 49 colored nongranular and 52 colored granular (table 4). Granular appears to be a simple recessive to nongranular and will be designated by the symbol gr.

When two recessives such as sp and gr both affect the same part of the plant, the question should be raised whether $sp\ gr$ plants constitute a different phenotype from $Sp\ gr$ or $sp\ Gr$ or whether they are indistinguishable from one of these types. Four families throw light on this question, but a discussion of two of them will be postponed until the question of linkage of these genes with C is discussed. Family 3013 arose from the cross $Cc\ oo\ Spsp\ Grgr \times CC\ OO\ spsp\ Grgr$ and segregated into 6 $COSpGr$: 8 $COspGr$: 3 $COSpgr$: 23 Co. If sp is epistatic, the expected frequencies of the first three terms of the ratio (excluding the Co

TABLE 4

FREQUENCIES OF COLORED (NONSPLOTCHED, NONGRANULAR), SPLOTCHED, GRANULAR, ORANGE, PALE-UPPER, AND BUFF TYPES IN ELEVEN FAMILIES SEGREGATING FOR SPLOTCHED, GRANULAR, OR BOTH

Family	Parents	Genotypes of parents				Colored	sp	gr	Orange	p	bu
3001	(1) (7)	aa	$Spsp$	$Grgr$	$Bu/$	8	1	2	4	7	0
3101	(6) (12)	aa	$Spsp$	$grgr$		6	14	10	14	8	0
3101	(6) (23)	aa	$Spsp$	$Grgr$ $\times$ Cc $Spsp$ s^vbu/s^vBu Pp		18	4	13	13	8	23
3103	(6) (23)	aa	$Spsp$	$grgr$ $Pp \times Cc$ $Spsp$ s^vbu/s^vBu		11	0	13	10	0	10
3105	(8) (23)	aa	$Spsp$	$Grgr$ s^vBu/s^vbu		9	0	12	3	0	6
3106	(22) (8)	Cc	$SpSp$	s^vBu/s^vBu $\times$ aa		9	0	10	16	0	0
3223	(3) (52)	Cc	$SpSp$	$Grgr$ s^vbu/s^vbu Pp		5	0	6	7	9	0
3225	(1) (5)	aa	$SpSp$	$Grgr$ s^vBu/s^vbu Pp		8	0	0	20	2	0
3226	(5) (3)	aa	$spsp$	$Grgr$ s^vbu/s^vbu Pp		31	0	13	7	0	0
3361	(52) (4)	aa	$Spsp$	$SpSp$ $Pp \times Cc$ $SpSp$ s^vBu/s^vbu Pp		7	0	8	5	3	1
3411	(4) (52)	Cc	$SpSp$	$grgr$ s^vBu/s^vbu Pp		5	0	0	13	0	0
3226	(7) (1)	Cc	$Spsp$	$Grgr$ $grgr$ s^vBu/s^vBu		7	0	13	13	0	0

types) are 6.4, 8.5, and 2.1, while the expected frequencies if gr is epistatic are 6.4, 6.4, and 4.2. In the first case, chi-square is 0.45, which—with three degrees of freedom—indicates a probability of 70–80%, while in the latter, chi-square is 0.77 and P is 50–70% (table 5). In family 3103, the cross is $cc\ Spsp\ grgr \times Cc\ spsp\ Grgr$ and the observed ratio was 6 colored : 14 splotched : 10 granular :

was merely the expression of the pale-upper gene on a C background, since the first family is segregating into 1 Gr : 1 gr and 1 P : 1 p, while the second is segregating into a 3:1 ratio in each case. However, a study of 3105 and 3106 (table 4) shows a segregation into 1 Gr : 1 gr, but no p plants. This would tend to show that gr and p are not the same genes. Similarly, in these two families there is a

TABLE 5

SEGREGATION INTO COLORED (C), SPLOTCHED (sp), AND GRANULAR (gr) ON ORANGE BACKGROUND, AND PROBABILITY ACCORDING TO EPISTASIS OF sp AND OF gr, AND ALSO (IN TWO FAMILIES) ACCORDING TO NO LINKAGE BETWEEN C AND sp AND ACCORDING TO LINKAGE WITH 20% CROSSING OVER. COLORED NONORANGE AND NONCOLORED TYPES NOT INCLUDED IN CALCULATION OF CHI-SQUARE VALUES

FAMILY*	OBSERVED					CHI-SQUARE VALUES			
						Linkage		No linkage	
	CO	sp	gr	Co	c	sp epistatic	gr epistatic	sp epistatic	gr epistatic
3001....	8	1	2	0	17	0.16 (90–95%)†	0.32 (80–90%)	1.68 (30–50%)	1.25 (50–70%)
3103....	18	4	13	0	44	0.87 (50–70%)	4.37 (10–20%)	29.05 (less than 1%)	4.45 (10–20%)
3013....	6	8	3	23	0	Cannot test linkage		0.45 (70–80%)	0.77 (50–70%)
3101....	6	14	10	0	22	Cannot test linkage		0.93 (50–70%)	7.60 (2–5%)

* Genotypes of parents.—

3001: $cSp/csp\ Grgr \times CSp/csp\ Grgr$
3103: $cSp/csp\ grgr \times CSp/csp\ Grgr$
3013: $Csp/cSp\ Grgr \times Csp/Csp\ Grgr$
3101: $cSp/csp\ grgr \times Csp/csp\ Grgr$

† Figures in parentheses indicate probabilities for two degrees of freedom.

22 noncolored. On the basis of the epistasis of sp, the expected ratio (excluding noncolored) is 7.5 : 15 : 7.5, but if gr is epistatic the ratio becomes 7.5 : 7.5 : 15. As shown from table 5, there is a much better agreement if it is assumed that sp is epistatic to gr.

The possible connection between the sp and gr genes and bu and p must be considered. Are either sp or gr merely the expressions of bu and p on a C background rather than on a c background? Families 3225 and 3226 might indicate that granular

segregation into 1 Bu : 1 bu (because of the elimination of s^y male gametes), which might indicate that gr and bu were identical; but the first two families disprove that, since they are segregating for gr but not for bu. Families 3361 and 3373 support these suggestions, since they are both segregating for gr but for neither p nor bu. There is some evidence also to indicate that sp is not identical with p or with bu. Family 3101 is segregating into 1 Sp : 1 sp but has no buff plant, while in 3411 there are bu but no

sp plants, although the population is small (table 4). In families 3223, 3225, 3226, and 3411 there are pale-upper but no' splotched plants, an indication that *sp* and *p* are not the same gene. There is no evidence regarding a possible connection between *sp* and *gr* and the yellow genes *y*, *gl*, and *ys*, since the first two were found only in orange families and the last three only in white.

With regard to linkage, there is probably no linkage between *gr* and the self-sterility alleles, since the *Gr* : *gr* ratios in the six families (3223, 3225, 3226, 3361, 3373, and 3411) that have a common self-sterility allele are not especially bad except for 3223 and the absence of *gr* plants in 3411. Since none of the families segregating for *sp* had a common self-sterility allele, nothing is known of possible linkage between *sp* and *s*.

Linkage with *y*, *gl*, and *ys* has not been tested, for *sp* and *gr* have not been found in *oo* plants. Linkage between *sp* or *gr* and *bu* or *p* cannot be tested, for it has not been possible to distinguish between *Sp* and *sp* and between *Gr* and *gr* in *cc* plants or between *Bu* and *bu* or *P* and *p* in *C* plants.

Since *c* is epistatic to *Gr* and *gr*, possible linkage between *gr* and *C* must be determined from the *Gr* : *gr* ratio in *C* plants in families which also throw recessive noncolored plants. The ratios in table 4 are generally sufficiently good so that there is no suggestion of linkage. The splotched gene, however, does appear to be linked with *C*, although the evidence is not so extensive as might be desired. Family 3004 arose from the cross *Cc Oo Spsp* × *Cc oo Spsp* and segregated into 20 colored orange non-splotched : 2 colored orange-splotched : 48 colored white : 10 noncolored orange : 15 noncolored white. The high percentage of whites in both the colored

and noncolored classes is not readily explained and is not concerned in the immediate problem. The segregation of the colored, orange plants into 20 nonsplotched : 2 splotched, however, is interesting. On the basis of independent assortment, the deviation divided by the standard error is 1.72, but if *C* and *sp* are linked in the coupling phase in each parent, with 20% crossing over, the expected ratio for the twenty-two plants is 19.4 nonsplotched : 2.6 splotched, and the ratio of the deviation to the standard error is only 0.40. The observed ratio therefore agrees somewhat better with that expected on the basis of linkage. Families 3001 and 3103 also segregate for both *C* and *sp* and could be used to test for linkage, but since they are also segregating for granular, the problem also involved the epistasis of *sp* over *gr*. It has previously been shown that in families 3013 and 3101 (table 5) the observed numbers of the self-colored, splotched, and granular types show a closer fit to the expected ratio based on the epistasis of *sp* over *gr* than that based on the epistasis of *gr*. In families 3001 and 3103, using chi-square, the observed ratios were tested against the expected ratio based on (*a*) the epistasis of *sp* and linkage between *C* and *sp* with 20% crossing over, (*b*) the epistasis of *gr* and such linkage, (*c*) the epistasis of *sp* and no linkage, and (*d*) the epistasis of *gr* and no linkage. In both cases the observed ratio is in closer agreement with the first assumption. While such an agreement does not prove the assumption, at least it can be said that in four families the ratios are consistent in favoring the assumption that *sp* is epistatic, and in three families the ratios favor the view that *sp* and *C* are linked with about 20% crossing over.

RED-OUTLINE (RO)

In the red-outline type, the anthocyanin pigmentation on the lower lip is generally confined to a thin red outline, although a few spots of red are also found near the tube, and on the splotched, red-outline type the splotched pattern may be present in addition to the outline. The upper lip is also red but the color is uneven (fig. 3). In some cases apparently the color is also greatly restricted in the upper lips, appearing only as a thin outline and a spot on each side of the lips near the tube. This gene apparently is expressed only in the presence of C.

Red-outline appears to be a recessive, although the data in regard to it are few. It is a difficult type to study because it is apparently affected by the presence of other genes. Unfortunately, the families in which it appeared were also segregating for a large number of genes which restrict the anthocyanin pigment to certain patterns, and in the course of following some of these other genes the red-outline type was neglected.

Family 3016 arose from a cross between two nonred-outline plants, 2901 (14) and 2901(16), and contained 11 nonred-outline and 6 red-outline. This looks like a simple recessive. Family 3005 was not so clear. The male parent was the same as in the previous family, but the female parent, 2901(7), appeared to be a red-outline phenotypically. Family 3005, however, consisted of 73 colored orange nonred-outline : 8 colored orange red-outline : 17 colored white nonred-outline : 2 colored white red-outline—all plants having anthocyanin pigment. The deficiency of red-outline cannot be explained. Family 3004 was from the cross 2901(7) × (13) and had 22 colored orange red-outline and 48 colored white nonred-outline. It is possible that both parents were recessive, although only

the female appeared to be red-outline phenotypically, and that some modifying gene prevented the expression of red-outline in the parent which had a white background. Family 3009 was from 2901(10), a red-flowered plant phenotypically nonred-outline, and 2901(5), a noncolored orange-flowered plant. The ten colored offspring from this cross were all red-outline, although the one parent was not red-outline phenotypically. Some of the discrepancies in these families may arise from the difficulty of scoring red-outline when certain other pattern genes are present, but it is also possible that red-outline may be caused by two different genes that produce a somewhat similar effect. Red-outline will be considered tentatively as a simple recessive and given the symbol *ro*.

BICOLOR (BI)

The bicolor type is another modification of the C type and is apparently not expressed in cc plants. It has been traced through several generations in the line of plants with a white background. In the colored nonbicolor plants, the inner part of the corolla limb is Pomegranate Purple or a related color, but in the bicolor type the upper lip is Pomegranate Purple and the lower lip is Rose Color. The lower part of the tube is orange with deep purple dots in both the nonbicolor and bicolor plants. There is no evidence whether the bicolor gene can act in colored plants that have an orange background. In one of the earliest families, several plants were described and catalogued which appear as if they might have been bicolors on a colored orange background, but unfortunately they were found in a family that was not used to produce subsequent generations. No known bicolors appeared in the orange line that was continued for a number of
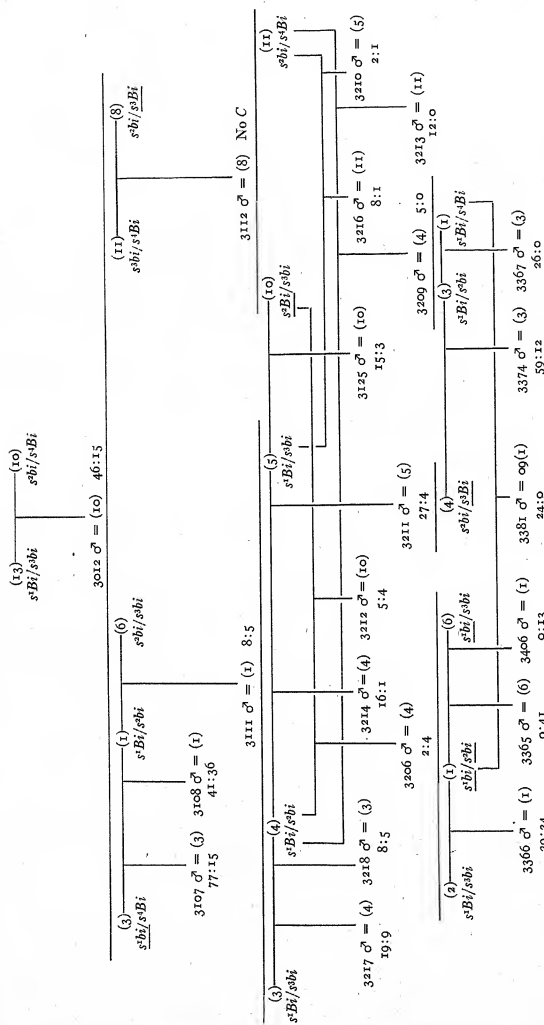
FIG. 4.—Pedigree diagram of families bearing on linkage between bicolor and self-sterility alleles. Ratios beneath pedigree numbers of families are ratios of non-bicolor to bicolor. Male parent of each family is indicated, as this is important when dealing with self-sterility. Crossover gametes indicated by a line beneath the s and bi genes.

generations. Crosses were made between bicolor plants on a white background and orange plants, but unfortunately the seeds of these crosses were no longer viable when they could be sown. This type has not been recovered in the new material as yet.

When two nonbicolor plants are crossed there are always nonbicolor plants in the offspring, although there are sometimes bicolors as well. Two families (3365 and 3406) arose from crosses between two bicolor plants. In one there were 41 bicolors and in the other there were 13, and in neither family was a nonbicolor found (fig. 4). A cross between a nonbicolor and a bicolor produced family 3381, which contained 24 nonbicolor and no bicolors. This last cross indicates that bicolor is recessive to nonbicolor.

Three families arose from a cross between two nonbicolors and contained both types of plants. In family 3012 there were 46 nonbicolors and 15 bicolors, while in families 3216 and 3210 the respective ratios of nonbicolor to bicolor were 8:1 and 2:1. In all three families and in the total of the three families the deviation from an expected 3:1 ratio was less than the standard error (table 6). One cross between a bicolor and a nonbicolor yielded 29 nonbicolored and 24 bicolored offspring (family 3366). On the basis of a 1:1 ratio the deviation in this family is 0.69 times the standard error. These families support the theory that bicolor is a simple recessive. The bicolor gene will be designated by the symbol bi.

Twelve other families arose from crosses between two nonbicolor plants and segregated into both nonbicolors and bicolors. As several showed a deviation more than twice as great as the standard error for a 3:1 ratio while some others had a deviation almost as large, it was

decided to consider the possibility that the bicolor gene might be linked with the self-sterility alleles. This possibility was strengthened by the fact that the families with disturbed ratios were from parents which had a self-sterility allele in common. The self-sterility alleles of the various plants used as parents of these families and the self-sterility classes of the families themselves were known (22), so the first step in the problem was to fit the Bi and bi genes into a logical coupling or repulsion arrangement which would produce the aberrant ratios of these families. When these families were plotted in a diagram (fig. 4), it was seen that in some instances the Bi gene must have been coupled with the common self-sterility allele in the male, while in other families the male parent must have had the bi on the same chromosome as the common s allele. Seven families appeared to fall into the first category and five into the second, and these two groups are listed separately in table 6. Of the first seven families, only one deviated significantly, but the group as a whole segregated into a ratio of 126 nonbicolor to 64 bicolor, with a deviation 2.6 times the standard error for a 3:1 ratio. If there is linkage between bi and s, these seven families when combined would indicate a crossing over of 33.68%. For the five families in which bi in the male is coupled with the common s gene, the combined ratio is 158 nonbicolor to 34 bicolor and the deviation is again significant. If linkage is assumed, crossing over is 35.42%. When these two groups of plants are considered together, linkage comes to about 34.6%.

Individually, few of these families deviate significantly from a 3:1 ratio, but when the observed ratios are compared with the expected 3:1 ratios and with the expected ratios based upon

TABLE 6

RATIO OF NONBICOLOR TO BICOLOR IN THREE FAMILIES WHICH DO NOT HAVE COMMON SELF-STERILITY ALLELE AND IN TWELVE FAMILIES IN WHICH ONE ALLELE IS COMMON TO BOTH PARENTS

FAMILY	GENOTYPES OF PARENTS	OBSERVED		EXPECTED (NO LINKAGE)			EXPECTED (LINKAGE WITH 34.6% CROSSING OVER)		
		Bi	bi	Bi	bi	Dev. S.E.	Bi	bi	Dev. S.E.
3012......	$s^0bi/s^3Bi \times s^0bi/s^1Bi$	46	15	45.75	15.25	0.07	......	......	......
3216......	$s^1Bi/s^0bi \times s^0bi/s^3Bi$	8	1	6.75	2.25	0.96	......	......	......
3210......	$s^0bi/s^4Bi \times s^1Bi/s^0bi$	2	1	2.25	0.75	0.33	......	......	......
Total....	......	56	17	54.75	18.25	0.34	......	......	......
3108......	$s^0bi/s^4Bi \times s^4Bi/s^0bi$	41	36	57.75	19.25	4.41	51.82	25.18	2.63
3217......	$s^4Bi/s^0bi \times s^4Bi/s^0bi$	19	9	21.0	7.0	0.87	18.84	9.16	0.06
3218......	$s^3Bi/s^0bi \times s^4Bi/s^0bi$	8	5	9.75	3.25	1.13	8.75	4.25	0.44
3212......	$s^3Bi/s^0bi \times s^4Bi/s^0bi$	5	4	6.75	2.25	1.35	6.06	2.94	0.75
3211......	$s^3Bi/s^0bi \times s^4Bi/s^0bi$	27	4	23.25	7.75	1.36	20.86	10.14	2.35
3214......	$s^3Bi/s^0bi \times s^4Bi/s^0bi$	16	1	12.75	4.25	1.82	11.44	5.56	2.31
3375......	$s^3Bi/s^0bi \times s^0bi/s^4Bi$	10	5	11.25	3.75	0.74	10.10	4.90	0.05
Total....	......	126	64	142.5	47.5	2.76	127.87	62.13	0.29
3107......	$s^4Bi/s^0bi \times s^0bi/s^4Bi$	77	15	69.0	23.0	1.93	76.08	15.92	0.25
3206......	$s^4Bi/s^0bi \times s^4Bi/s^0bi$	2	4	4.5	1.5	2.36	4.96	1.04	3.18
3215......	$s^4Bi/s^0bi \times s^4Bi/s^0bi$	15	3	13.5	4.5	0.82	14.89	3.11	0.06
3209......	$s^0bi/s^3Bi \times s^4Bi/s^0bi$	5	0	3.75	1.25	1.29	4.14	0.86	1.02
3374......	$s^0bi/s^3Bi \times s^4Bi/s^0bi$	59	12	53.25	17.75	1.57	58.72	12.28	0.09
Total....	......	158	34	144.0	48.0	2.33	158.78	33.22	0.15

linkage between bi and s with 34.6% crossing over, it is found that the agreement with the latter ratio is better in nine of the twelve families. In two families (3211 and 3214) the observed ratio agrees better with the expected $3:1$ ratio. The reasons for this are not clear, but these families are from reciprocal crosses and some other factor may be operating. The third family (3206) contains only six C plants, deviates significantly from both expected ratios, and probably should not be included.

Only one family resulted from a cross between a heterozygote and a bicolor. This was family 3111, whose parents were 3012(6) and 3012(1). Family 3012 arose from two of the original plants and segregated into a perfect $3:1$ ratio. The parents had no self-sterility alleles in common, for four self-sterility classes were found in 3012. Plant 3012(6) was s^2s^3 and a bicolor. Plant 3012(1) was s^1s^2 and heterozygous. When this plant was crossed as a male on to 3012(3) to produce family 3108, it appeared that the Bi gene must be coupled with the s^1 gene as the ratio in 3108 could not otherwise be explained. If plant 3102(1) is s^1Bi/s^2bi, the s^2 gametes should be eliminated in the cross 3012(6) $\times$ (1), and there should be an excess of Bi plants over the expected $1:1$ ratio, all bicolors appearing merely as the result of crossing over. Only thirteen C plants were raised in this family, and eight were nonbicolor and five were bicolor. On the basis of no linkage the deviation is 0.83 times the standard error, but on the basis of linkage it is only 0.29 times the standard error. While neither ratio deviates significantly, again there is better agreement with the theory based on linkage.

Even though a series of disturbed ratios fits into a scheme based on linkage with self-sterility alleles, the possibility

that the morphological genes are eliminated for some other reason must also be considered. Linkage with Ga genes, such as MANGELSDORF and JONES (16) found in maize, with complete elimination of the ga gametes and 34.6% crossing over between Ga and bi, could also explain some of these disturbed ratios. To determine whether linkage with such genes is the cause of the gametic elimination of Bi or bi genes, the ratio of the two self-sterility classes in both the Bi and bi phenotypes should be examined. EAST and MANGELSDORF (7) showed that when the two parents have a common self-sterility allele, the self-sterility class of the mother is always absent in the progeny, and that, of the two classes in the offspring, one is identical with that of the paternal parent while the other is different from the classes of both parents. If Ga genes were operating, the ratio of Bi to bi would be determined by the nature of the coupling of Ga and bi. Half the Bi plants would belong to the paternal self-sterility class and half to the nonparental class, while half the bi plants would be of the paternal class and the other half would be nonparental. If bi was linked with the s genes, the ratio of paternal to nonparental classes would not be $1:1$ for both the bicolor and nonbicolor types, but it would be different for each. For example, if Bi in the male were coupled with the common self-sterility allele, the paternal Bi plants would be more numerous than the nonparental Bi type, while more nonparental bi plants than paternal bi's would be expected. Likewise, if bi were coupled with the common allele in the male, the nonparental nonbicolors should exceed the paternal, while there should be more paternal than nonparental bicolors. Table 7 lists the total of these four groups for three families in which Bi was coupled in

the male and for four families in which bi was coupled in the male. While chi-square is not significant for either theory, it is smaller in each case when the observed ratio is tested against the expected, based on linkage of bi and s, than when tested against a possible linkage of bi with Ga genes and absence of linkage between bi and s. This test affords additional evidence to suggest that the gene for bicolor is linked with the self-sterility alleles.

These families do not segregate for C and therefore contain all colored plants. Since there are no white plants, the ratio of nonbicolor to bicolor should be $3:1$ if the disturbed ratios in segregating families are due to linkage between bi and C rather than between bi and s. These two nonsegregating families support the theory that bi is not linked with c. This idea is further supported by family 3012, which is segregating into colored and noncolored plants but in which the ratio

TABLE 7

COMBINED RATIOS OF NONBICOLOR TO BICOLOR AND OF PATERNAL TO NONPARENTAL SELF-STERILITY CLASSES. FOLLOWING THE OBSERVED RATIOS ARE (1) EXPECTED RATIOS IF THE GAMETIC ELIMINATION OF Bi AND bi IS NOT THE RESULT OF LINKAGE BETWEEN BICOLOR AND SELF-STERILITY ALLELES AND (2) EXPECTED RATIOS IF bi AND s ARE LINKED WITH 34.6% CROSSING OVER

LINKAGE IN MALE PARENT	NO. OF FAMILIES	OBSERVED				EXPECTED (1)					EXPECTED (2)				
		Non-bicolor		Bicolor		Non-bicolor		Bicolor		X^2	Non-bicolor		Bicolor		X^2
		P*	N	P	N	P	N	P	N		P	N	P	N	
Bi coupled.......	3	12	9	2	5	9.4	9.4	4.6	4.6	2.24	10.8	8.0	3.2	6.0	0.87
bi coupled........	4	14	17	7	2	16.5	16.5	3.5	3.5	4.54	15.4	17.6	4.6	2.4	1.47

* P, paternal; N, nonparental.

As in the case of the buff gene (25), since bi cannot be tested in cc plants, and since linkage between bi and C would therefore give disturbed ratios of Bi to bi in C plants of families segregating for C, the possibility that the disturbed ratios in these families are due to linkage between bi and C rather than between bi and s must be considered. If linkage were between bi and C, the ratios should not be disturbed in families which do not segregate for C. In families 3107 and 3108 (table 6) the ratios are very much nearer those based on linkage between bi and s than those based on no such linkage. In fact, in 3107 the deviation from the expected $3:1$ ratio is highly significant while in 3108 it is almost significant.

of nonbicolor to bicolor is a perfect $3:1$ ratio.

Since bi appears to be linked with s, it should also be linked with buff, as the latter is closely linked with s. This possible linkage cannot be tested directly, however, since bu is expressed only in cc plants, while bi is expressed only in plants containing the dominant allele C. For a similar reason, it is not possible to test linkage between bicolor and pale-upper, but since bi is linked with s while p is not, bi and p cannot be linked. For a similar reason, bi cannot be linked with O and is probably not linked with y, gl, ys, or gr. It is not known whether sp and s are linked. No data are available to test linkage between bi and sp, since the

bicolor plants were found only in the *oo* strain while the splotched type was identified only in *O* plants.

CHIMAERAS

Chimaeras throw some light on the recessive nature of noncolored, just as they did for nonorange and for yellow (**25**). Several have been found. Three plants were scored as Carmine and were in families in which at least some of the plants were heterozygous for *C*. In each plant there was an orange streak in the lower lip. One other plant from one of these same families had several orange streaks in both lips. In one plant of a family segregating for *C* on a white background, there was a white streak on the upper lip which was otherwise Pomegranate Purple. In one flower of a plant of family 3372, a more complicated chimaera was found. This plant was a carmine-flowered plant on an orange background. The color was uneven, but as the family was composed of several different pattern types it was not certain whether the irregularity in color was due to *sp*, to some other pattern gene, or to both. It had one wide chimaera in the center of the lower lip. This streak was Pomegranate Purple, but on each side of it there was a narrow streak of white. Apparently there was considerable mitotic disturbance when the flower was developing, resulting in the loss of the *O* gene at one point and the loss of *C* at two other points within the area that had lost the *O*.

The presence of orange streaks in red flowers and white streaks in purple flowers supports the idea that in the absence of some confusing, modifying genes, *CO* is red and *Co* is purple. Two plants from a family homozygous for *O* were Ox-blood Red but had one flower with several white chimaeras on the upper lip. At first sight these appear to be contradictory to the general rule, but a close examination showed that within the white sectors the entire epidermis was missing, so that the white streaks represented subepidermal tissue.

Summary

1. Colored flowers are dominant over white and are the result of gene *C*, a gene for anthocyanin pigmentation. In general, this gene in combination with *O* produces Carmine or Ox-blood Red flowers, while *C-oo* plants are usually Pomegranate Purple, Amaranth Purple, or Rose Color. Certain modifying genes vary the color, and in some plants it is impossible to determine from the color of the anthocyanin whether the background is white or orange. Gene *C* does not appear to be linked with the self-sterility alleles, or with *O*, *bu*, *p*, *y*, *gl*, or *ys*.

2. Two of the important modifiers of *C* are the splotched (*sp*) and granular (*gr*) genes. Both are recessives and are expressed only in *C* plants. In the splotched type the color is uneven and frequently found in irregular blotches and streaks. This gene is variable in its expression. In granular, there are numerous small colorless dots in the anthocyanin region, and again the expression of the gene is not uniform. Apparently neither *sp* nor *gr* is merely an expression of *bu*, *p*, *y*, *gl*, and *ys* on a *C* background. Several families indicate that *sp* is epistatic to *gr* and that *sp* and *C* are linked with about 20% crossing over.

3. Red-outline (*ro*) is another modification of *C*. The pigmentation tends to be restricted to an outline on the margin of the lower lip or of both lips, although other areas of color are usually present also on the lips. It appears to be a simple

recessive, but the data are meager and some ratios are poor. Apparently it is hypostatic to *c*.

4. Bicolor (*bi*) is a modification of *C* in which the anthocyanin color is considerably paler on the lower lip. This has appeared with certainty only in the strain homozygous for *o*, but there is some indication that it also affects *CO* plants. In general, the upper lip is Pomegranate Purple and the lower lip Rose Color. It does not seem to have any expression in *cc* plants. It is linked with the self-sterility alleles, with about 34.6% crossing over.

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INTERACTION OF NITROGEN NUTRITION AND PHOTOPERIOD AS EXPRESSED IN BULBING AND FLOWER-STALK DEVELOPMENT OF ONION

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Introduction

Photoperiod has been shown (2, 3, 5, 6) to exercise strong control over bulb formation in onion. Long photoperiods promote bulbing, and short ones delay, or—if sufficiently short—completely inhibit it. The level of nitrogen nutrition has also been found (1, 9) to influence bulb formation, high levels delaying bulbing and low levels accelerating it. The effects of these two factors on the flowering of onion have received much less attention, but knowledge of such effects would be helpful to onion breeding and to an understanding of the photoperiodic reactions involved. Previous studies have not simultaneously varied the nitrogen nutrition and the photo-

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period, so such experiments have been undertaken here in order to determine whether any interactions exist, and, if so, what their effect is on bulbing and flowering.

Material and methods

These experiments were conducted in the greenhouse at various times of the year. The first experiment reported did not deal with different levels of nitrogen nutrition, and the plants were initially grown in soil in 4-inch pots. Later they were transplanted to soil benches. The plants for the other two experiments were grown in sand in $1\frac{1}{2}$-liter glazed crocks. In these tests the concentration of available nitrogen was varied.

In the nutrition experiments, eight solutions (table I) were used. Solutions 1, 2, 7, and 8 were employed in experiment II and solutions 3–7, inclusive, in

experiment III. Solution no. 1 differed from all others in both the form and the concentration of the major elements, but solutions 2–8 were identical in composition, except for their concentrations of nitrogen. All solutions were complete except no. 2, which contained no nitrogen. A modified Hoagland solution (4) was employed to supply the minor elements in each nutrient solution. The solutions were applied daily in sufficient quantity to insure adequate flushing from the crock drains.

Results

Experiment I

The plants for this experiment were produced from hybrid onion bulbs[4] (F_2 population of male-sterile Italian Red 13–53 × Red Wethersfield) planted in soil in pots October 29, 1941. They were grown until December 19 in controlled environment rooms,[5] where they received 12-hour photoperiods and four different combinations of night and day temperature. Following this, they were

TABLE 1

Composition of Nutrient Solutions

SOLUTION NO.	MOLAR CONCENTRATION OF C.P. SALTS						N (P.P.M.)
	$MgSO_4 \cdot 7H_2O$	$CaCl_2 \cdot 2H_2O$	KH_2PO_4	$(NH_4)_2SO_4$	$Ca(NO_3)_2 \cdot 4H_2O$	$NaNO_3$	
1...............	0.0023	....	0.0023	0.0007	0.0045	....	73
2...............	.0058	0.0030	.0015	....	....	....	0
3...............	.0058	.0030	.0015	....	....	0.0002	3
4...............	.0058	.0030	.0015	....	....	.0009	13
5...............	.0058	.0030	.0015	....	....	.0018	26
6...............	.0058	.0030	.0015	....	....	.0028	39
7...............	.0058	.0030	.0015	....	....	.0037	52
8...............	0.0058	0.0030	0.0015	....	....	0.0075	105

Photoperiods both longer and shorter than those occurring naturally were employed. Supplemental light from 100-watt incandescent filament bulbs was used to extend the natural photoperiods. In experiments I and II the intensity of supplemental light was 40–50 foot-candles and in experiment III about 25 foot-candles at pot level.

The plants requiring photoperiods shorter than those occurring naturally were either covered with a double thickness of black sateen cloth or moved into dark chambers after they had received the desired duration of light. The data were subjected to variance analysis (8) and the significance of differences determined by the t-test.

moved to the greenhouse, transplanted to soil benches, and subjected to three different lengths of photoperiod until April 10, when the experiment was terminated.

The four temperature treatments applied while the plants were in the control rooms consisted of 65° F. night and day, 45° F. night and day, and both night and day combinations of these two temperatures. The photoperiods employed after the plants were moved to the greenhouse

[4] All onion plant material was supplied by Dr. Henry Jones, Bureau of Plant Industry Station, Beltsville, Maryland.

[5] A complete discussion of the features of these rooms has recently been reported by Parker (7). The light is secured from a combined carbon-arc Mazda source and is of a quality similar to sunlight.

were 10, 14, and 18 hours. Each photoperiodic treatment included plants from all the four temperature treatments, and the experiment was set up in duplicate in adjoining greenhouse sections.

Frequent dissections were made of plants from the various treatments, beginning December 19 when they were moved to the greenhouse, but the first inflorescence primordia were not observed until January 16. Flower stalks first became macroscopically visible by the middle of February, and by April 10

TABLE 2

INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON PRODUCTION OF FLOWER STALKS BY HYBRID ONION PLANTS. SIXTEEN PLANTS PER LOT

TEMPERATURE (° F.)		NUMBER OF FLOWER STALKS DEVELOPED ON PHOTOPERIODS		
Light period	Dark period	10-hour	14-hour	18-hour
65	65	4	10	7
65	45	4	12	11
45	65	2	11	14
45	45	8	8	12

(when the experiment was terminated) they were present on 103 of the 192 plants. The remaining eighty-nine plants were dissected at that time, and only three contained inflorescence primordia. The temperature variables to which the plants were subjected during the first weeks of growth did not result in any statistically significant flowering differences (table 2), but the plants that had received no low temperature during their early development produced fewer flower stalks than did those of the other three treatments. The greatest differences in flowering were associated with differences in photoperiod. About equal numbers of plants on 14- and 18-hour photo-

periods produced flowers, but less than half as many flowered on 10-hour photoperiod, and at the time the experiment was terminated these were in a less advanced stage of development than those of the longer photoperiods. On many of the 10-hour plants the bract that normally envelops the inflorescence grew into a long, curved, green structure resembling a vegetative leaf blade. The 14-hour plants exhibited the same character to less degree, but it was not observed in the 18-hour lots. Representative plants from the 10-, 14-, and 18-hour photoperiods are shown in figure 1.

EXPERIMENT II

On July 21, 1942, two closely related plant lines, 74 and 75, were planted outdoors in soil in thumb-pots. These lines had been derived from Yellow Bermuda × White Persian F_1's, backcrossed once to Yellow Bermuda and then selfed. The germinated seedlings were subjected to 8-hour photoperiods for 6 weeks and then were washed free of soil and transplanted to quartz sand in glazed crocks in the greenhouse. During the following 2 weeks, while still on 8-hour photoperiods, the plants were given a complete nutrient solution (solution 7; table 1) supplying 52 p.p.m. of nitrogen. Experimental treatments involving two lengths of photoperiod and four nutrient solutions differing mainly in their nitrogen content were begun at the close of this period, on September 28.

Eight treatment blocks were established, four each for the 11- and the 13-hour photoperiods. Each block consisted of four rows of ten plants. One continuous half of each row contained plants of line 74 and the other half plants of line 75, the relative position of the plant lines alternating from row to row.

Four nutrient solutions (1, 2, 7, and

8; table 1) were used in each block, a different one for each row. Solution 1 supplied nitrogen in the form of both nitrate and ammonia at a combined rate of 73 p.p.m., while solutions 2, 7, and 8—which contained no ammonia—supplied nitrate nitrogen at rates of 0, 52, and 105 p.p.m., respectively.

These treatments were continued until November 2, 1942, at which time all plants that had received the minus-nitrogen solution had begun to bulb on both photoperiods, with the most advanced bulbing on the longer photoperiod. Bulbing was not evident in any other treatment on either photoperiod. At this time all plants of both photoperiods that had been receiving 105 p.p.m. of nitrogen were placed on minus nitrogen, and those that had been receiving minus nitrogen were placed on 105 p.p.m. of nitrogen. By January 1, 1943, the plants that had been shifted from minus to high nitrogen no longer showed any evidence of bulbing; however, the lots that had been shifted from high to minus nitrogen were in an advanced stage of bulb development. This reciprocal transfer demonstrated that a high level of nitrogen would suppress bulb formation even after the process was initiated, and that withholding nitrogen would promote bulbing even in plants that had previously been grown on a high nitrogen level.

By January 14, all plants except those on the highest nitrogen level had begun to bulb, but none had approached maturity. At this time the bulb diameter of each plant was recorded, and the means for these measurements are shown in table 3. Since none of the bulbs were mature, these diameters indicate only the relative stages of development attained at the time the measurements were made. The plants maintained on minus nitro-

gen were characterized by erect, nonsucculent, pale green leaves; those supplied with 105 p.p.m. of nitrogen had declining, succulent, dark green leaves. Those
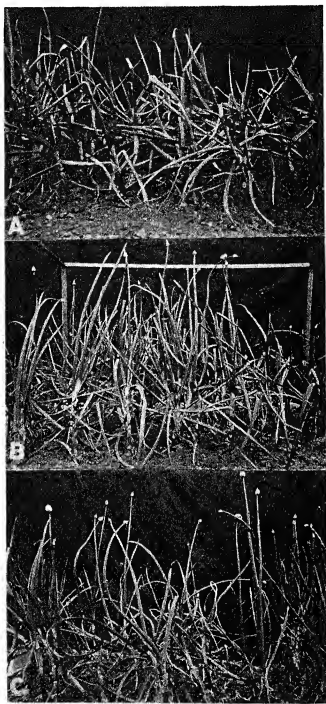
FIG. 1.—Hybrid onion plants grown on photoperiods of A, 10 hours; B, 14 hours; C, 18 hours. Photographed April 3, 1942.

of the other two solutions were intermediate in these characteristics and also in their degree of bulbing.

Differences in bulb diameter signifi-

cant at the 1% level were associated with photoperiod, solution, and replication, but not with plant line. The F-values of photoperiod and solution were each more than twice the F-value of replication. There was significance at the 5% level for the interaction of photoperiod and solution but not for any of the remaining two-factor interactions. This is interpreted to mean that the progressive increase in difference between the two photoperiods that occurred as the nitro-

PHOTO-PERIOD (HOURS)	PLANT LINE	MEAN DIAMETERS (CM.) OF BULBS GROWN IN VARIED NITROGEN CONCENTRATIONS*			
		105 p.p.m.	73 p.p.m.	52 p.p.m.	0 p.p.m.
13......	75	1.6	2.6	2.8	3.6
	74	1.7	2.8	2.5	3.2
11......	75	1.4	2.1	1.9	2.5
	74	1.4	2.0	1.9	2.6

* Diameter difference of 0.6 cm. required for significance at 1% level.

gen content of the solution was decreased was significant.

Under both daylengths (table 3) the greatest degree of bulbing was associated with the minus-nitrogen level, while the least degree was associated with the highest level. Bulbing was intermediate in the other two treatments, and—although part of the nitrogen was supplied in the form of ammonia—for one of these treatments there was no significant difference in the stages of bulb development under the two treatments. With each nitrogen treatment the degree of bulbing was greatest under the longer daylength.

The most outstanding effect of the nitrogen factor is evident (table 3) in the degrees of bulbing on the 11-hour photoperiod as against those on the 13-hour. Plants receiving a minus-nitrogen solution and an 11-hour photoperiod exhibited a greater degree of bulbing than did those receiving the 105 p.p.m. nitrogen solution and 13-hour photoperiod. Statistically, this difference is highly significant.

The data in table 3 indicate no apparent difference between the bulbing of the two plant lines under comparable experimental treatments. This behavior was to be expected, since the parental stocks from which these two lines came had been selected for uniformity with respect to bulbing performance.

Single flower stalks were visible on each of three plants in the experimental population when the bulb diameters were recorded on January 14, so the experiment was continued until March 27, to demonstrate any effects of the various treatments upon flowering. No change was made in the nutrient treatments during this time but one hour was added to each photoperiod, making them 12 and 14 hours each. During the 10-week period, daily records were taken of emergence of flower stalks of individual plants.

The most obvious result of this part of the experiment was the difference in flowering response of the two lines when subjected to the shorter photoperiod (table 4). All but one of the eighty plants of line 75 flowered, but only twenty-three of line 74 flowered under this condition. The remaining fifty-seven plants of line 74 were not dissected; it is possible that the inflorescence primordia may have been present in some of them. This difference in flowering response between the two lines with the shorter pho-

toperiod is great enough to be highly significant.

A similar but less obvious difference appeared between the two lines on the longer photoperiod. Ten plants of line 75 failed to flower but all of these had formed mature bulbs. In line 74 fourteen plants failed to flower but only six of these had formed mature bulbs. Thus, all the seventy non-bulbing plants of line 75 flowered, but eight of the seventy-four non-bulbing ones in line 74 failed to

TABLE 4

INFLUENCE OF PHOTOPERIOD AND NITROGEN NU-
TRITION ON PRODUCTION OF FLOWER STALKS
OF ONIONS FROM SISTER PLANT LINES. TWENTY
PLANTS PER LOT

PHOTOPERIOD (HOURS)		PLANT LINE	NO. FLOWER STALKS PRODUCED BY PLANTS GROWN IN VARIED NITROGEN CONCENTRATIONS			
Sept. 28–Jan. 14	Jan. 14–Mar. 27		105 p.p.m.	73 p.p.m.	52 p.p.m.	0 p.p.m.
13.......	14	75	19	16	19	16
		74	16	18	18	14
11.......	12	75	19	20	20	20
		74	3	4	9	7

flower. This difference between the two lines may not be statistically significant but it is in the same direction as the greater difference found on the shorter photoperiod.

Although there was no significant difference in number of plants of line 75 flowering under the two photoperiods, there was considerable difference in the degree of flower-stalk development. The inflorescences of plants in the lot on the longer photoperiod were considerably more advanced than those on the shorter photoperiod. Figure 2, showing representative long-day and short-day treatment blocks, illustrates this difference in

degree of development of flower stalks and umbels. Figure 3 shows individual
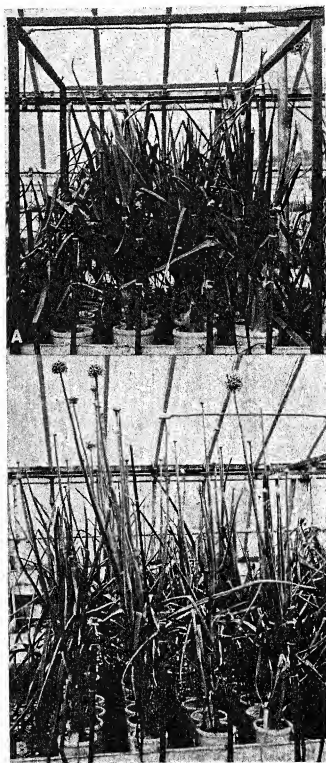
FIG. 2.—Onion plants of sister lines grown on photoperiods of A, 12 hours; B, 14 hours. Photographed March 13, 1943.

plants representative of the types developed in certain of the treatments at the close of the experiment.

EXPERIMENT III

Uniform sets, $\frac{3}{8}-\frac{1}{2}$ inches in diameter, of Ebenezer onion were planted in quartz sand in crocks in the greenhouse. The crocks were set on six trucks, fifty pots per truck, so that they could be readily moved to adjacent dark rooms. Ten

14 hours were maintained on pairs of trucks throughout the experiment. The daily duration of natural light was the same for all lots and varied from $8\frac{1}{2}$ to 10 hours as the season progressed. This period of natural light was extended by incandescent light of approximately 25
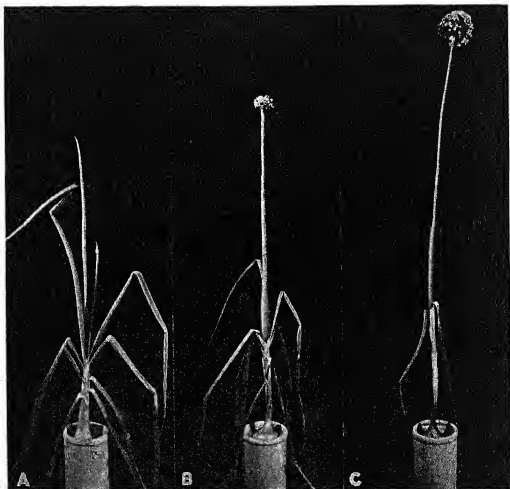
FIG. 3.—Onion plants grown with different photoperiods and with different concentrations of nitrogen: A, 12 hours and 52 p.p.m. nitrogen; B, 12 hours and 0 p.p.m. nitrogen; C, 14 hours and 0 p.p.m. nitrogen. Photographed March 13, 1943.

plants on each truck received one of five nutrient solutions daily (solutions 3–7; table 1). These solutions differed only in concentration of nitrogen. Although the relative balance of major-salt ions was not constant for all five solutions, the only plant symptoms evident during the experiment occurred in the low-nitrogen lots and could be ascribed to a deficiency of nitrogen. Photoperiods of 10, 12, and

foot-candles to give the desired photoperiod.

The treatments were started January 7, 1943, when the sets were planted, and the first leaf blade had emerged from all sets by January 16. The plants were grown on the several photoperiods and nitrogen treatments for 8 weeks, ending March 17. At this time some of the plants on the 14-hour photoperiods had devel-

oped bulbs that were nearly mature, so the experiment was terminated. The roots were discarded and the rest of the plant was divided into, leaf-base and leaf-top portions. The leaf-base part of some of the plants was a definite bulb but in others varied from a slightly swollen to an unswollen pseudostem. In the former case the leaf tops were cut just above the bulb and in the latter they were cut 1 inch above the basal plate. The tissue was dried in a forced-draft oven at 80° C. for 48 hours and the dry weight of each fraction determined.

The dry weight of bulb or pseudostem expressed as percentage of the total dry weight of the plant was used as a measure of the degree of bulbing. The mean values of these percentages, as well as the mean dry weight per plant for the various treatments, are given in table 5.

All the plants on 14-hour photoperiod bulbed, regardless of the level of nitrogen supplied, and those from the four solutions of highest nitrogen composition attained about the same average weight. Although the bulbs from the solution of 3 p.p.m. of nitrogen were little more than half as large as the rest, they reached maturity at approximately the same time. The bulbs of all lots contained about the same percentage of the weight of the whole plant.

While all plants bulbed on the 14-hour photoperiods, only twelve of the 100 plants on 12-hour photoperiod showed marked bulbing. Five of the latter were grown with 3 p.p.m. of nitrogen and the remaining seven with 13 p.p.m. In the 10-hour treatments there were no well-developed bulbs, but in some lots the plants had begun to swell slightly at the base. The data on degree of bulbing for plants of the 10- and 12-hour lots (table 5) indicate only the relative development attained at the time of harvest.

Bulbing differences between plants of the 14-hour and those of either the 12- or the 10-hour lots were obviously significant. Accordingly, an analysis of variance was limited to the data on degree of bulbing of the 12-hour and 10-hour lots. Such data show significance at the 1% level for nitrogen concentration but not for photoperiod or replication. There is no significance shown for any two-factor interaction.

TABLE 5

EFFECT OF PHOTOPERIOD AND NITROGEN SUPPLY ON DRY WEIGHT OF ONION PLANTS AND DEGREE OF BULBING.* DATA BASED ON MEAN OF TWENTY PLANTS PER LOT

CONCEN-TRATION OF NITRO-GEN (P.P.M.)	DRY WEIGHT (GM.) OF PLANTS GROWN ON PHOTOPERIODS			DEGREE OF BULBING ON PHOTOPERIODS		
	10-hour	12-hour	14-hour	10-hour†	12-hour†	14-hour
52.......	2.7	2.8	1.8	12.5	13.4	68.8
39.......	2.5	1.8	2.0	16.9	15.2	66.0
26.......	1.9	1.9	1.7	17.0	16.6	64.5
13.......	1.8	2.0	1.9	17.0	18.9	66.0
3.......	0.7	0.7	1.0	22.9	22.8	69.0

* Degree of bulbing = $\dfrac{\text{Dry weight of bulb or pseudostem}}{\text{Dry weight of total plant}}$ × 100.

† Difference of 5.9 required by the nitrogen treatments for significance at 1% level.

Discussion

GARNER and ALLARD (2), McCLELLAND (6), WILSON (9), and MAGRUDER and ALLARD (5) have indicated the dependency of onion bulbing upon the relative length of day. In all varieties investigated they report that bulbing occurred under the longer photoperiods and was inhibited or delayed by the shorter ones. WILSON, one of the first to study the effects of nutrition on the bulbing of onion, investigated the relation of nitrate nitrogen to the carbohydrate and nitrogen content of onion plants grown

either in sand culture or soil. He noted that excessive quantities of nitrate tended to depress total yields of bulbs without affecting the yield of tops. Since photoperiod was not a variable in this particular experiment, WILSON was unable to observe any interactive effects of nitrogen nutrition and photoperiod.

BREMER (1) was the first to note that earlier bulbing of onion could be obtained through a nitrogen deficiency. He completed both greenhouse and field experiments, but like WILSON, he used but one photoperiod in any one experiment. The development attained at harvest time was indicated by expressing the weight of bulbs as a percentage of the total plant weight. On this basis, BREMER reported earlier bulbing if the plants received a deficient level of nitrogen—either in soil or in sand culture.

The data presented in this paper substantiate the fact that length of photoperiod is a controlling factor in onion bulbing. Plants grown with the duration of photoperiod well above a certain critical range bulb immediately, and those grown below do not. This critical range may vary somewhat for different varieties, but within this range the process of bulb formation is slower than it is with longer photoperiods, and the plants are more susceptible to the influence of other environmental factors. This is illustrated by the data of experiments II and III. The plants in experiment II were grown near the critical range of photoperiod for bulbing, and nitrogen nutrition affected the degree of bulbing. In experiment III the longest photoperiod was farther from the critical range, and nitrogen nutrition did not influence the bulbing response. Thus, an interaction of photoperiod and nitrogen nutrition on bulbing was evident when the plants were grown near the critical photoperiod.

Under these conditions a deficient level of nitrogen has the same effect as lengthening the photoperiod, and a high nitrogen supply has the same effect as shortening the photoperiod.

It is well recognized that varieties of onions differ in bolting habit. Experiment II shows that photoperiod may be one of the controlling factors in the flowering response of onions. One of the two strains of experiment II showed rather striking differences in flowering between the two photoperiods employed, while the other showed only slight differences. Low temperature during the development of the plant is known to be a most important factor in determining whether onions will flower, but these data indicate that photoperiod may also play a prominent role.

The difference in flowering response on short photoperiod of these two closely related strains of onion was not unexpected. The two strains were derived from parent stocks selected for uniformity of bulbing; no particular attention had been given to selection for uniformity of bolting. By using controlled photoperiods such selections could be readily made, if desired.

Plant breeders desirous of producing successive generations in the shortest time possible can grow their onions from seed to seed without an intervening dormant period if the environment is correctly manipulated. If this is to be done, the seedling should be started and maintained on a photoperiod below the critical for bulbing. After the plants have grown for several months with relatively low temperatures, the photoperiod can be lengthened to promote flowering but must not be lengthened so much that the plants will produce mature bulbs without flowering. High concentrations of nitrogen added at this time help to de-

press the bulbing tendency resulting from the longer photoperiod. The data of this study do not indicate that such nitrogen applications interfere with floral development.

Summary

1. A study was made of the several effects of photoperiod and nitrogen nutrition upon the bulb and flower-stalk development of onion. Plants were grown from seed, sets, and mature bulbs, and with soil and in sand culture.

2. Plants grown on photoperiods substantially longer than that critical for bulb formation showed no difference in bulb development when supplied different amounts of nitrogen.

3. Plants grown on a photoperiod at or near that critical for bulb formation exhibited the greatest bulb development with the lowest nitrogen concentration and the least with the highest concentration.

4. Under certain environmental conditions nitrogen and photoperiod can interact to influence bulb development of certain onion varieties.

5. Onion plants of sister lines gave similar bulbing response on photoperiods of identical duration. However, the two lines differed in their production and development of visible floral stalks, especially on the shorter of the photoperiods. Development of floral stalks was favored by long photoperiods.

6. In a hybrid line unrelated to the sister lines, long photoperiods also favored flower-stalk development, but low temperatures applied during the early growth of these plants were without measurable effect.

7. Onion plants were grown from seed to seed without an intervening dormant bulb stage by controlling the photoperiod and concentration of nitrogen available for growth.

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HISTOLOGICAL CHANGES IN BINDWEED AND SOW THISTLE FOL-
LOWING APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC
ACID IN HERBICIDAL CONCENTRATIONS[1,2]

H. B. TUKEY, C. L. HAMNER, AND BARBARA IMHOFE

Introduction

Recent investigations have called at-
tention to a new principle in herbicidal
action following application of 2,4-di-
chlorophenoxyacetic acid to bindweed,
sow thistle, and other plants (4, 5, 9).
Most other herbicides, such as sodium
chlorate and sulphuric acid, depend upon
caustic properties to destroy the parts of
the plant with which they come into im-
mediate contact. The action of 2,4-
dichlorophenoxyacetic acid, however, is
physiological (11); the chemical, or its
effect, travels some distance through the
plant to induce a telemorphic response
(2) which contributes to the death of the
plant. The typical gross symptoms fol-
lowing application to bindweed and sow
thistle are curvature and chlorosis of
above-ground parts and proliferations of
below-ground parts. The external evi-
dence is an indication of changes taking
place within the plant. This paper pre-
sents a study of these responses on a cel-
lular level, to determine, if possible, how
they contribute to the death of the plant.

Material and methods

The plants used for anatomical and
histological study were bindweed (*Con-
volvulus arvensis* L.) and sow thistle
(*Sonchus arvensis* L.). They were grow-

ing in a cultivated area in the immediate
vicinity of Geneva, New York, the sum-
mer of 1944. The soil of the area is char-
acteristically heavy, has a pH of 6.5, and
is provided with a fairly uniform mois-
ture supply.

The plants were sprayed with an aque-
ous solution of 2,4-dichlorophenoxy-
acetic acid at 1000 p.p.m. in a carrier of
0.5% Carbowax 1500, using a knapsack
sprayer. Bindweed was treated on July
14 and 24, 1944, when mean tempera-
tures were approximately 80°–85° F. by
day and 55°–60° F. by night, and when
plants were in a condition of vigorous
growth (4, 5). Material for histological
study was collected on July 21, 24, and
25. Flower buds, leaves, stems, under-
ground stems, and roots were gathered.
The underground parts were obtained to
a depth of 14 inches, at which depth the
main horizontal roots are found (3),
since the watertable at Geneva is rela-
tively high. Vigorous plants of sow thistle
were treated on September 12, when
mean temperatures were approximately
70°–80° F. by day and 55°–60° F. by
night. The crown and 6 inches of vertical
underground stem were collected on
September 21 for histological study.

All material was fixed in formalin-
acetic-alcohol, and the butyl alcohol-
paraffin method of dehydration and im-
bedding was used. Sections were stained
either with safranin and light green or
with Flemming's triple stain modified by
Margolena to include iodine.

Results

BINDWEED

FLOWER.—In gross appearance 1 day after treatment, the petals, stamens, and pistils of open flowers were brown, and anthers were shrunken and distorted. Flower buds and unopened flowers were arrested in development and failed to open.

The histological characteristics of the flower are similar to those of other members of the Convolvulaceae and need not be detailed here. Unopened flowers were used, since they provided unshed pollen as well as other floral parts still in development. The cells of the ovary wall were parenchymatous, and those in the developing ovules were meristematic. The cells of the nucellus were active and filled with starch grains. Starch was also found in moderate amounts in the sepals, petals, anthers, and pollen grains.

Histological changes were pronounced in the unopened flowers 7 days after treatment. The cellular structure of the sepals and petals seemed not affected, but the starch reserve, which was present in moderate amounts in those tissues, had disappeared by that time. Ninety-five per cent of the pollen grains had been affected, the cytoplasm having shrunken frequently from the walls—as if severe plasmolysis had occurred. A characteristic feature was the appearance of large vacuoles, as contrasted with smaller ones found in the pollen of untreated plants (fig. 1). Development of the endothecium of the anther was arrested. The cells appeared plasmolyzed, starch had disappeared, and cell contents were greatly reduced in density. In the ovule the nucellar tissue immediately surrounding the gametophyte differed markedly in untreated plants, the cytoplasm being much less dense. All meristematic activity in the nucellus had apparently ceased, and the cells had the appearance of mature parenchyma. Starch had disappeared from the entire ovule, except in the innermost layers of the nucellus. The cells of the ovary wall, although re-
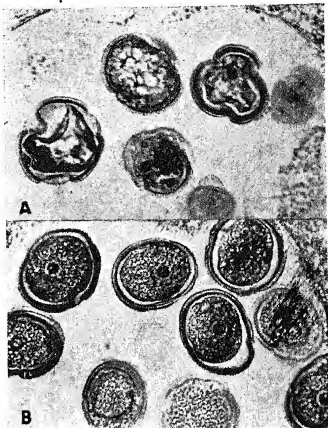
FIG. 1.—Effect of 2,4-dichlorophenoxyacetic acid on pollen grains of bindweed: *A*, pollen from treated plants, showing plasmolysis and large vacuoles. *B*, from untreated plants, showing turgid condition of pollen grains, and small vacuoles.

maining more typically parenchymatous, underwent similar reduction in starch content.

LEAF.—Within a few hours after treatment, the leaves in gross appearance were wilted and slightly folded upward along the midrib. After 24 hours the symptoms were more strongly evident, the plants having become dull green in color and lying flat to the ground. By the fifth day the basal leaves were yel-

low, and by the tenth day all leaves were withered and dead.

The leaves of untreated plants require no special mention, except that palisade cells occur on both the dorsal and ventral sides of the leaf. Minute grains of starch were observed in the chloroplasts.

Seven days after treatment the cells of the mesophyll and of both dorsal and ventral palisade layers had largely lost their dense contents and had become contracted, narrow and irregular in

TABLE 1

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS IN STEM OF BINDWEED, SEVEN DAYS AFTER TREATMENT

TISSUE	AVERAGE DIAMETER OF CELLS (μ)		APPROXIMATE NO. OF ROWS OF CELLS	
	Un-treated	Treat-ed	Un-treated	Treat-ed
Epidermal layer.....	21	24	1	1–2
Cortex.............	52	53	11	11
Phloem region......	15	15	6	10
Cambial zone.......	12	12	2–4	3–5
Internal phloem.....	15	12	5	8
Pith...............	42	66	9	10

shape, and plasmolyzed. Chloroplasts were somewhat smaller and fewer in number than in untreated plants, but no reduction in starch content was observed. Many newly divided cells were found in the phloem region of the principal vascular bundles.

STEM.—The principal gross responses of the stem to treatment were arrest in development, limpness, and loss of characteristic twining habit. Within 24 hours many climbing stems were hanging loosely, and others were lying flat to the ground.

The typical stem has a heavily cutinized epidermis. The cortex consists of a broad band of somewhat flattened cells containing scattered latex vessels which remain active throughout the life of the plant (7). At the inner part of the cortex is a conspicuous band of starch-filled cells, including the endodermis. Immediately within the endodermis is an intermittent ring of pericyclic fibers. A band of external secondary phloem is separated from the xylem by the cambial zone. The secondary xylem forms a complete or almost complete cylinder several layers in thickness, immediately adjacent to which are numerous islands of internal phloem. The two regions are separated by an intermittent band of internal cambium.

Response following treatment was varied. The epidermal and cortical cells were not greatly affected, although some cell division occurred in the subepidermal layer (table 1). The endodermal cells were activated to some extent, and both anticlinal and periclinal divisions were observed. The phloem and cambial regions showed activity within 24 hours of treatment, cell division occurring in both regions by that time. By 7 days after treatment the phloem region had become greatly activated and had increased in radial width 66% (table 1). The primary parenchyma had become crushed, and the pericyclic fibers were distorted and compressed and no longer stood out as a distinct ring. The cells of the cambial zone had also undergone stimulation by that time and had formed a multi-layered band around the stem approximately twice as wide as in untreated stems. The mature xylem cells seemed not to have been affected. Internal phloem, however, underwent a change characteristic of treatment in both stem and rhizome. No longer serving as a storage region, the areas of phloem became actively dividing tissue. The meristematic

cells in these areas, being small, contrasted sharply with the larger parenchymatous cells of the pith, in which there was considerable enlargement of individual cells but no disorganization of the tissue (table 1).

RHIZOME.—The vertical rhizomes of bindweed develop from buds which are borne on the extensive horizontal root system. They are typically light-colored and somewhat woody and contain a great deal of storage material.

The gross response of the rhizome to treatment was in marked contrast to that of the above-ground parts. Five days after treatment, rhizomes were spongy, water-soaked, and enlarged to twice the diameter of similar portions of untreated plants. The outer layers showed longitudinal splitting and were easily sloughed off. The buds which are borne on the rhizomes failed to develop into shoots.

The structure of the typical untreated rhizome resembles that of the above-ground stem, although there is a much greater amount of secondary phloem and xylem, and pericyclic fibers are not found in the deeply buried portions of the rhizome (7). Sections cut 1–2 inches below the ground level contain only a few scattered fibers. The xylem is composed of a wide and almost continuous ring of large vessels, interspersed with small, thick-walled cells.

A considerable amount of starch is found in the rhizome, located mainly in the inner portion of the cortex. Parenchymatous cells of this region contain an abundance of large starch grains. Very little starch occurs in the outer portion of the cortex. The pith and ray cells contain a small amount of starch, although the individual grains are very small.

The response to treatment was more pronounced in the rhizome than in any

other part of the plant (fig. 2). Individual cells of the periderm layer did not increase in size, but some cells in the subepidermal layer divided periclinally, resulting in a region three to four cells in thickness as compared with the 2- to 3-celled zone in untreated plants (table 2). The periderm, however, was ruptured and split in many places.

The cells of the outer portion of the cortex were greatly enlarged (table 2) and the walls frequently torn. The region increased in width radially from approximately 0.35 mm. to 0.60 mm. Pericyclic fibers, where present, were crushed and somewhat displaced. Except for the occasional fibers, there was no clear demarcation between the inner portions of the cortex and the phloem.

Starch disappeared gradually from the inner part of the cortex following treatment and had almost completely disappeared within 7 days (fig. 3). The grains which remained were clustered around the nuclei of the cells (fig. 3C). In some cases, however, starch disappeared after 1 day. This may have been due to the rapid action of the chemical, under optimum conditions.

Meristematic activity was markedly stimulated in the outer phloem and cambial zone. The phloem region increased radially from approximately 0.15 mm. to 0.30 mm. in width. A wide band of small active cells appeared after treatment, as shown in figure 2. In material collected 7 days after treatment, the cells of this entire activated zone contained dense cytoplasm and large nuclei, and they were easily distinguished from the adjacent cortical cells in which nuclei were small and often not seen in cross-section because of the large size of the cells.

Longitudinally, cells of the activated phloem region of treated plants were ar-
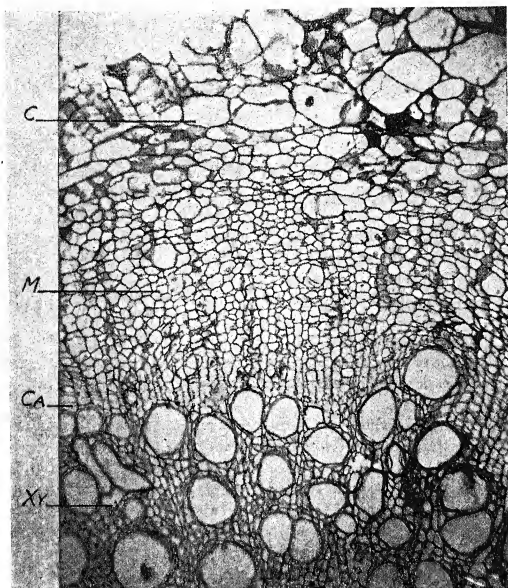
Fig. 2.—Typical response of rhizome of bindweed to treatment with 2,4-dichlorophenoxyacetic acid, showing activated meristematic region (*m*) external to several-layered cambial zone (*ca*) and xylem (*xy*) and enlarged cells of cortex (*c*).

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS AND ON RADIAL WIDTHS IN RHIZOME OF BINDWEED, SEVEN DAYS AFTER TREATMENT

REGION	AVERAGE DIAMETER OF CELLS (μ)		NO. OF ROWS OF CELLS		RADIAL WIDTH OF REGION (mm.)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Periderm.........	43	43	3–5	3–5	0.1	0.15
Cortex...........	74	124	6–10	9–10	0.35	0.6
Phloem region.....	31	22	10–17	18–26	0.15	0.3
Xylem vessels......	452	529	5–6	5–6	0.45	0.6
Internal phloem....	18	16	10–12	8–14	0.1	0.2
Pith.............	47	68	4–5	4–6	0.25	0.3

ranged very regularly, and the typical long sieve tubes were few. This condition might indicate horizontal divisions in the phloem similar to those reported in *Iresine* after treatment with indoleacetic acid, in which long rows of cells were derived from a single parent phloem cell (6).

The internal phloem was also stimulated. As seen in cross-section, groups of phloem cells had formed clearly outlined, bulging, dome-shaped areas which developed inward, corresponding to similar areas in the stem. In the rhizome there were usually three main groups and one or two smaller ones, but the groups themselves were larger and more pronounced than those of the stem, almost completely filling the pith region. The pith cells immediately adjacent to these areas were compressed, so that a distinct layer of two or three rows of crushed cells often outlined the regions (fig. 4).

In general, the cells of the rhizome, especially those in the activated phloem region, were characteristically cytoplasmic and turgid, in sharp contrast to the cells of the flowers and leaves, which were highly plasmolyzed and vacuolate and showed no indication of becoming part of an actively dividing tissue.

ROOT.—The gross response of the root was similar to that of the rhizome but of less intensity. The roots increased somewhat in diameter and became spongy and water-soaked in appearance, accompanied by splitting and sloughing off.

The root has an outer corky tissue several layers in width. The root is an important storage organ, and the phelloderm and cortex comprise the main storage regions. The cells of the cortex especially are filled with many large starch grains. Within the cortex is a band of secondary phloem which contains many latex vessels (7). The xylem forms a solid cylinder in the center of the root

FIG. 3.—Effect of 2,4-dichlorophenoxyacetic acid on starch in rhizome of bindweed: *A*, portion of region in untreated rhizome showing abundance of starch grains in cortex and phloem regions. *B*, comparable area in treated region in which starch has entirely disappeared except for minute grains in one or two isolated cells. *C*, detail of cell showing the few remaining starch grains clustered around the nucleus.

and is composed of large vessels and many tracheids.

Histological changes in the main horizontal roots, 14 inches below the surface of the soil, were evident 7 days after treatment but were relatively small in comparison with changes in other por-

It is possible that activity in the roots would have continued for some time after the date of collection.

SOW THISTLE

Sow thistle is a perennial weed reproducing by means of seeds and creeping
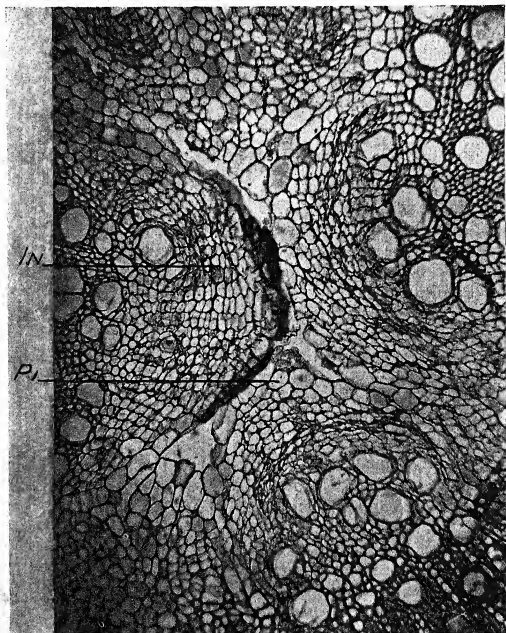
FIG. 4.—Detail of rhizome of bindweed treated with 2,4-dichlorophenoxyacetic acid, showing central region in which localized activity of internal phloem (*in*) has occurred, causing crushing in pith (*pi*).

tions of the plant. By that time the small parenchymatous cells near the large xylem vessels had divided, the cambial zone had become active, and the starch in nearby cells had partially disappeared.

roots. The horizontal roots, brittle and yellowish, grow in the upper 2–4 inches of the soil. A few thickened vertical roots may penetrate 5–10 feet. The rhizomes develop from buds on the horizontal

roots and terminate in rosettes of light green leaves at the ground level.

The gross responses to treatment were rapid. Within 24 hours the leaves had become lighter green in color and slightly wilted. Two days after treatment the younger leaves were severely twisted and curled. After 10 days the outer leaves

RHIZOME.—The typical rhizome is composed largely of parenchymatous cells (fig. 5B). At a point 1 inch below the crown of the plant, where sections were made, the periderm layer is three to four cells wide. The cortex is a narrow band of parenchyma, eight to twelve cells in width, containing a few scattered
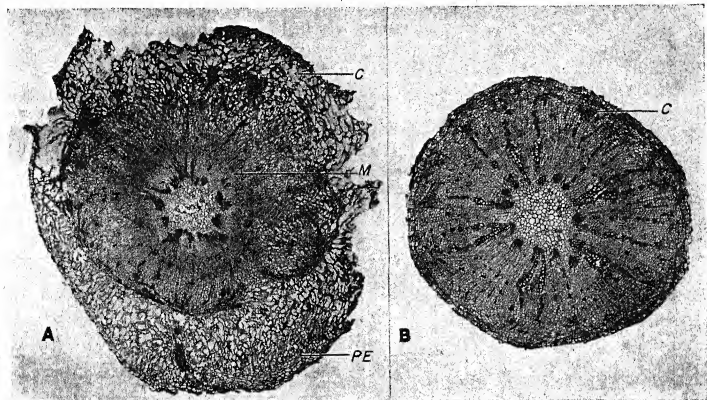
FIG. 5.—Typical response of rhizome of sow thistle to treatment with 2,4-dichlorophenoxyacetic acid: A, rhizome showing increase of cortex (c) to several times usual size, due to cell enlargement; formation of secondary periderm (pe) in cortex; and loss of identity of strands of secondary phloem in activated region (m) within intermittent ring of compressed latex vessels and endodermis. B, rhizome from untreated plant, showing relatively small cortex (c) and distinct radial strands of xylem and phloem.

were progressively lighter green and decidedly wilted, while the central leaves showed extreme curvature. The bases of the inner leaves were enlarged and flattened, resembling the petioles of celery. The most conspicuous response was the large increase in diameter of the rhizome, ranging from 50% to 300%. All underground parts were soft and spongy and exhibited marked splitting of the outer regions. Entire plants were dead 2 weeks after treatment.

latex vessels. The endodermis, pericycle, and phloem region together constitute approximately one-fourth of the radius of the rhizome. The endodermis is intermittent and indefinite, so that the parenchyma of the inner region appears to be continuous with that of the cortex. Differentiated phloem occurs in narrow radial strands separated from one another by relatively broad bands of parenchyma. Lactiferous ducts and groups of medullary phloem which are in close con-

junction are scattered throughout the region. The xylem constitutes nearly one-third of the radial diameter and, like the phloem, occurs in radial strands between which are broad bands of parenchyma. The pith is relatively small, being approximately one-fifth the radius of the rhizome.

There was much disorganization in most of the tissues of the rhizome, owing both to an increase in, and stimulation of, cellular activity and to the enlargement of cells (fig. 5A). The periderm was

less degree than in the cortex. The regions increased in radial width from 0.5 mm. to 0.8–1.0 mm. In the outer part of the phloem region many cells had doubled in diameter, and the latex cells tended to become indefinite in form. Toward the inner part of the region there was much cell division but no pronounced cell enlargement. The greatly increased activity of the cambial zone and phloem region resulted in a disorganized mass of dividing tissue, and the identity of the radial strands of phloem was completely lost.

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SIZE AND NUMBER OF CELLS AND ON RADIAL WIDTHS IN RHIZOME OF SOW THISTLE, NINE DAYS AFTER TREATMENT

REGION	AVERAGE DIAMETER OF CELLS (μ)		NO. OF ROWS OF CELLS		RADIAL WIDTH OF REGION (mm.)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Periderm...........	62	68	3–6	2–4	0.1	0.1
Cortex............	58	128	8–12	10–12	0.2	0.9–1.4
Pericycle and phloem region...........	21	17–54	18–22	26	0.5	0.8–1.0
Cambial zone.......	20	16	3–4	3–5	0.15–0.2	0.25
Xylem vessels......	169	135	16–20	19–20	0.5	0.4
Pith..............	55	58	8–10	8–10	0.5	0.6

ruptured in many places, and cells of the cortex were greatly enlarged, especially in a radial direction, to two to ten times the usual width (table 3). Individual cells occasionally measured approximately 200 × 300 μ, as compared with approximately 20 × 50 μ in an untreated plant. The cortical region as a whole increased in width from approximately 0.2 mm. to 0.9–1.4 mm. The resulting increased size of the cortex became the most conspicuous feature of treated plants. Secondary periderm layers were occasionally formed in the disorganized cortex.

Cells of the pericyclic and phloem regions were distorted, although to a much

Many lateral roots observed in treated material may have been initiated by the treatment. The pith was relatively less affected than other portions of the rhizome, although there was occasional rupturing of the tissue.

STARCH.—A striking and characteristic response to treatment was the disappearance of starch grains in portions of the flower, stem, rhizome, and root. With the exception of the flower, starch disappearance was correlated with new growth of cells. Starch grains were fissured, angular, and smaller in size in storage cells nearest the regions of stimulated activity. In the flower, starch disappeared from the petals, anthers, and ovary in the ab-

sence of cell division, although its disappearance may have been correlated with the development of tissues along other lines. The leaves did not show pronounced acceleration of starch digestion after treatment, although the chlorotic appearance of the leaves indicated that the function of the chloroplasts had been impaired.

Since treatment in most instances promoted the disappearance of starch from

taka-diastase. A temperature of 25° C. was maintained during the experiment. The color change in each test tube was recorded with a Lovibond colorimeter at 1-minute intervals up to 10 minutes.

The results given in table 4 fail to show any acceleration of starch hydrolysis with the acid beyond the normal rate of hydrolysis of the control. On the contrary, at 100 mg./l, and especially at 1000 mg./l, there was marked inhibition

TABLE 4

EFFECT OF VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON HYDROLYSIS OF STARCH WITH TAKA-DIASTASE, AS MEASURED BY LOVIBOND COLORIMETER. LOSS OF COLOR* INDICATES HYDROLYSIS OF STARCH

CONCENTRATION	COLOR	No. OF MINUTES AFTER INTRODUCTION OF TAKA-DIASTASE										
		0	1	2	3	4	5	6	7	8	9	10
Control.........	Red	3.2	3.1	2.9	2.4	2.2	1.8	1.7	1.5	1.3	1.3	1.2
	Blue	5.0	4.5	4.2	3.4	3.2	2.7	2.5	2.4	2.2	2.0	1.9
1 mg./l.........	Red	3.0	2.8	2.2	2.0	1.7	1.4	1.4	1.4	1.2	1.1	1.0
	Blue	5.5	4.8	3.8	3.3	3.1	3.1	3.1	3.1	2.9	2.3	2.1
10 mg./l.........	Red	2.6	2.4	2.3	2.1	1.9	1.8	1.4	1.3	1.3	1.2	1.2
	Blue	4.6	4.3	3.8	3.5	3.2	3.0	2.9	2.6	2.6	2.2	2.2
100 mg./l.........	Red	3.2	3.1	2.4	2.4	2.2	2.1	2.1	1.5	1.4	1.4	1.4
	Blue	5.4	5.0	4.8	4.2	3.7	3.3	3.2	3.1	3.0	2.9	2.7
1000 mg./l......	Red	3.2	3.2	3.2	3.2	3.2	3.2	3.1	3.1	3.1	3.1	3.1
	Blue	5.5	5.3	5.2	5.2	5.2	5.2	5.1	5.1	5.0	4.9	4.7

* Numbers indicate intensity of color.

the cells of living plants, tests were made of the effect of 2,4-dichlorophenoxyacetic acid on starch *in vitro*. For this purpose, 1% starch solution was prepared with Argo cornstarch. Ten cubic centimeters of the solution was placed in each of five test tubes: one remained as a control, while the acid was added to each of the other four in concentrations of 1 mg./l, 10 mg./l, 100 mg./l, and 1000 mg./l. One-tenth cubic centimeter of iodine solution (0.3% iodine and 1% potassium iodide) was added to each test tube. This was followed by 5 cc. of a 1% solution of

of hydrolysis. This same reaction might not necessarily occur in the plant, however, as there are many other factors involved in plant processes.

Discussion

Many of the responses induced by the action of 2,4-dichlorophenoxyacetic acid on bindweed and sow thistle resemble those reported by KRAUS *et al.* with indoleacetic acid on bean (8). This acid, however, produces a much greater effect in more dilute concentrations, and it also produces a marked response at some dis-

tance from the point of application, traveling a considerable distance in the underground system. The distance that the acid travels is important, since regeneration of the plant may occur from underground buds if these are not injured.

The primary object of the present study was to determine the histological changes which accompanied death of bindweed and sow thistle following treatment with 2,4-dichlorophenoxyacetic acid and to attempt to correlate these changes with the death of the plants.

In the case of bindweed, the effect of the acid on immature pollen grains and on developing tissues of the ovule becomes important, in view of the propagation of the plant by seeds. When 95% of the pollen becomes functionless, the probability of fertilization is greatly reduced, the arrested floral development precluding the development of seeds.

The chlorotic appearance of the sprayed leaves suggests that the manufacture of chlorophyll in the chloroplasts is impaired, and the division of phloem cells of the larger vascular bundles of the leaf suggests a possible disturbance in the normal movement of food from the leaf.

Increased cell division, which was an early symptom of response, especially in the rhizome, occurred first and was greatest near the large xylem vessels. This fact might indicate that the acid or some substance stimulated by it was transported through the plant in these vessels. As in the leaf, the phloem of stem and rhizome—being in close proximity to the xylem elements—underwent marked stimulation, which might easily result in a major disturbance in the transfer of food. Such a condition would be of vital importance to the life of the underground regions, where no food is manufactured.

An increase in meristematic activity also indicates an increase in the rate of respiration and the further depletion of reserves, which has been shown to be an important factor in weed control (1, 10).

The enlargement and proliferation of cells in the rhizome were accompanied by disorganization and rupturing of cells and tissues. The tearing of the epidermis and periderm in many places provided an easy means of entry for fungi and bacteria. Since starch was found to disappear from many regions, there is the possibility of an increase in available sugars, which would be favorable to the growth of organisms and to the final decay and disintegration of the plants.

In a general sense, the changes in the rhizome of sow thistle were not unlike those in the rhizome of bindweed. The greatly increased cell division in the phloem and cambial regions and the marked increase in size of cells of the cortex resulted in disorganization and rupturing of cells and tissues, which not only markedly alter the appearance but also upset the normal functions of the plant, and so offer opportunity for microörganisms to bring about disintegration and decay.

Summary

1. Bindweed and sow thistle were treated with aqueous sprays of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. in 0.5% Carbowax 1500, applied during midsummer while the plants were growing vigorously. The responses of the plants were studied and the tissues examined to correlate histological changes with the death of the plants.

2. In bindweed, pollen grains were plasmolyzed and disorganized, flowers were arrested in development, chlorophyll formation was checked, and cell division was activated in the large vascular bundles of the leaves. The majority of the cells in the leaves were plasmolyzed.

3. Cell division was greatly increased in all cambial zones and phloem regions of the stem and rhizome of bindweed. Enlargement and rupture of cortical cells of the rhizome were conspicuous features.

4. The root of bindweed responded more slowly to treatment than did other parts of the plant, so that changes which had occurred 7 days after treatment were of less intensity than in the rhizome but similar in nature.

5. Starch disappeared from almost all parts of the flower of bindweed, but very little hydrolysis of starch occurred in the chloroplasts of the leaves. Disappearance of starch from the endodermis of the stem and the inner cortex of the rhizome and root was correlated with active cell division in the phloem region of those portions of the plant.

6. In the rhizome of sow thistle, cells of the cortex were greatly enlarged and frequently torn. The periderm was ruptured, and disorganized large-scale cell division occurred in the cambial zone and phloem regions.

7. Starch hydrolysis was inhibited *in vitro* by the action of the acid.

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DEVELOPMENT OF SPORE-FORMS AND THE NUCLEAR CYCLE IN THE AUTOECIOUS OPSIS RUST, CYSTOPSORA OLEAE

M. J. THIRUMALACHAR

Introduction

The principal diagnostic characters of the genus *Čystopsora*, founded by BUT-LER (9) to accommodate the rust on *Olea dioica* Roxb., are the occurrence of basal cells or cysts in the telia and the germination of the teliospores without a rest period. The telia are superstomal, and the teliospores—which germinate *in situ*—form a semi-internal, 2-celled promycelium, each promycelial cell bearing a sessile sporidium. BUTLER found a few aecia associated with the telia, and he tentatively interpreted them as the aecial stage of the rust. AJREKAR (1), who carried out infection tests, confirmed the interpretation of BUTLER and showed that the rust is an autoecious opsis form. *Cystopsora oleae* Butl. has been reported from Ceylon by PETCH (21) on *Olea polygama* Wight, and SYDOW (24) has recently established a second species, *C. notalaeae*, on *Notalaea longifolia* Vent. from Australia.

Abundant living material of this rust became available in 1940 at the Coffee Experiment Station, Balehonnur, Mysore State, and a careful examination of it showed that the promycelia, instead of being 2-celled, were in fact 4-celled. This provided the stimulus for undertaking a detailed study in order to follow the various nuclear phases of the species. The investigation has revealed that not only are the promycelia 4-celled, but that the sequence of life cycles is very unusual. In fact, the type of development is so remarkable, and so much at variance with accepted concepts regarding the function and the sequence of the various spore-

forms, that it has been considered worth while to place on record all the various carefully made observations in the course of this investigation. Opportunity has also been taken to emend the diagnosis of the genus and the species, to conform to the new facts now brought to light.

METHODS.—Material for the study was fixed in three fluids—Bouin's as modified by Allen, Craf, and 2BD. Heidenhain's iron-alum haematoxylin with eosin in clove oil was generally employed for staining the spores; but for critical studies, especially when the nuclear phase of the mycelium and of the spores had to be confirmed, Feulgen's stain was used. Delafield's haematoxylin and safranin combinations were employed with good effect for studying the pathological anatomy.

Observations

SPORE-FORMS

The rust occurs on the host throughout the year in one or the other spore-form. Pycnia and aecia are distributed on the leaves and tender shoots, which become much hypertrophied. When the axillary buds are infected, especially their meristem, large "witches' brooms" studded with pustules are formed. On their being shaken, clouds of aeciospores are shed. Later the witches' brooms dry up but the mycelium perennates in the basal region. When fresh growth takes place, the newer outgrowths are completely covered with the sori.

PYCNIA.—These are subepidermal and flask-shaped, with well-developed ostio-
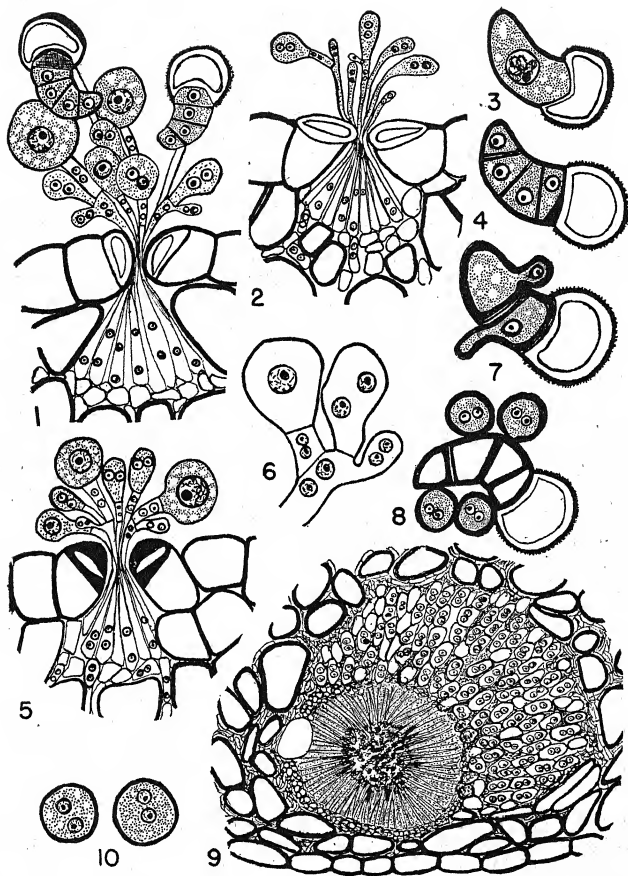
FIGS. 1–10.—Figs. 1, 5, section through telium. Fig. 2, through young telium. Figs. 3, 4, germination of teliospores. Fig. 6, development of teliospores in clusters. Fig. 7, 2-celled promycelium. Fig. 8, germinating teliospore with sessile sporidia. Fig. 9, differentiation of pycnium and aecium from same plectenchyma. Fig. 10, binucleate sporidia.
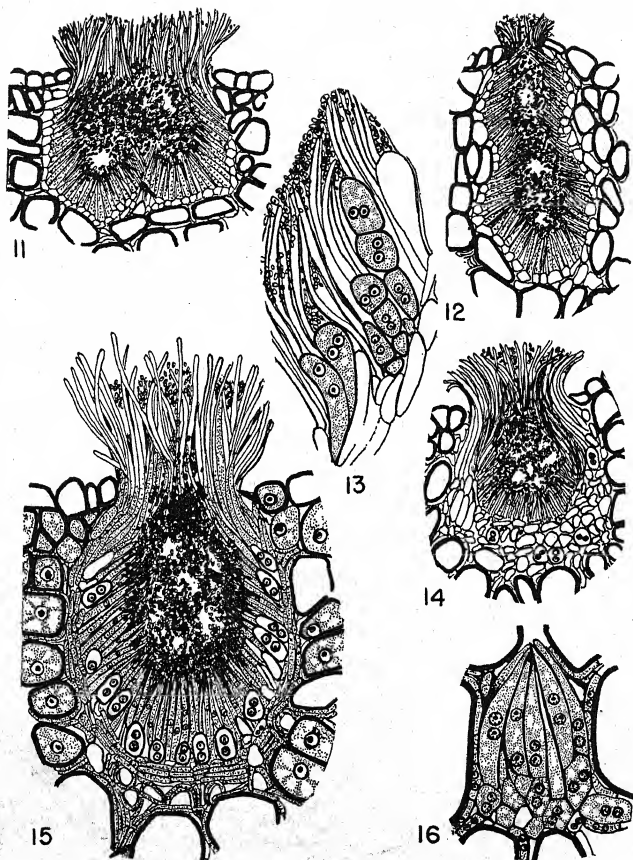
FIGS. 11–16.—Fig. 11, coalescing pycnia. Fig. 12, coalescence of superposed pycnia. Fig. 13, abstriction of aeciospores from basal cells beneath pycnium. Fig. 14, development of plectenchyma within pycnium. Fig. 15, subepidermal pycnia. Fig. 16, binucleate pycnial hyphae and basal cells.

lar filaments (fig. 15). They are amphigenous and can be differentiated from the aecia by their orange-yellow color. They occur in abundance, both on the leaves and on the hypertrophied portions of the witches' brooms but are soon displaced by the aecia. When two pycnia occur close together, they coalesce (fig. 11), and in some cases they may even become superposed in such a manner that the ostiole of the lower pycnium fuses with the basal region of the one above, resulting in the formation of a continuous chamber (fig. 12). In such cases the spermatia from both pycnia emerge through a common ostiole.

AECIA.—These are amphigenous, erumpent, and cupulate. They are yellow, in contrast to the orange-yellow color of the pycnia. No peridium surrounds them. The aeciospore chains, like the pycniosporophores, converge toward the center, however, rather than form vertical rows (fig. 21). This type of formation of aeciospore chains has evidently eliminated the necessity of a peridial wall. When in close proximity, the aecia may also coalesce, and the fusion of aecial cups that are superposed has been noted (fig. 27). Aeciospores are yellow, binucleate, ovate-elliptic to fusiform, deeply angular, with distinct germ pores. The germ pores become rather prominent at the time of germination. Their exospore is sculptured with reticulations which become distinct when the spores are mounted in plain agar. They can be germinated without difficulty and stained by the method suggested by the writer (26).

TELIA.—The telia are hypophyllous, pulverulent, and sparsely distributed on the undersurface of the leaves. When close together they also coalesce, forming concentric rings. They emerge through the stomatal aperture (figs. 1, 5), but the infection patches are not hypertrophied.

The hyphae passing out of the stoma are distinctly separate (fig. 2), and this feature is observable in the early stages. The tip of the emerging hypha becomes rounded off, and a binucleate spore initial is cut off from which the stalk cell and the teliospore initials are fashioned. By repeated division the basal cell forms successive spore initials, which in turn form more stalk cells and teliospores (fig. 6). The teliospores are therefore formed in basipetal succession by the activity of a single generative cell. When a branch is separated by manipulation under the microscope, clusters of spores at different stages of maturity and attached to the basal generative cell can be clearly observed. In a mature telium the basal cell becomes expanded, forming a discoid apex. It is manifest that BUTLER mistook the fascicular basal cells for cysts and, on this basis, discussed the affinities of the genus *Cystopsora* with *Ravenelia*, *Uromycladium*, and others where the development of cysts is a well-established character. In fact, the structure referred to by BUTLER is the discoid basal generative cell. In this respect the genus shows affinity toward *Chaconia* or *Scopella*—genera in which the teliospores develop in clusters from laterally free generative cells. The young teliospores are binucleate, but later a syncaryon is formed (fig. 17). The mature teliospores are minutely verrucose and very much thickened, but only over the distal two-thirds of their circumference. The proximal third is smooth and thin, and the stalk is inserted into the thin portion—usually laterally.

GERMINATION

The teliospores germinate within the telium as soon as they are mature and while still attached. There is no germ pore, but the proximal thin-walled por-
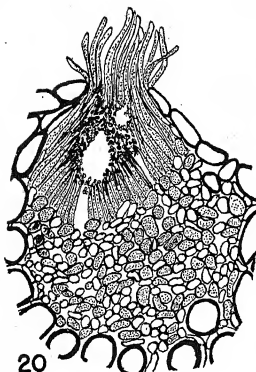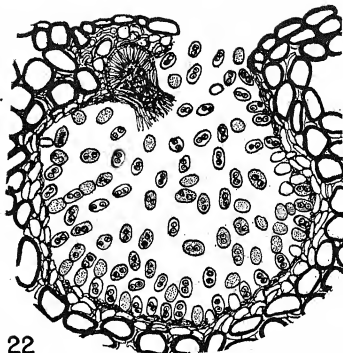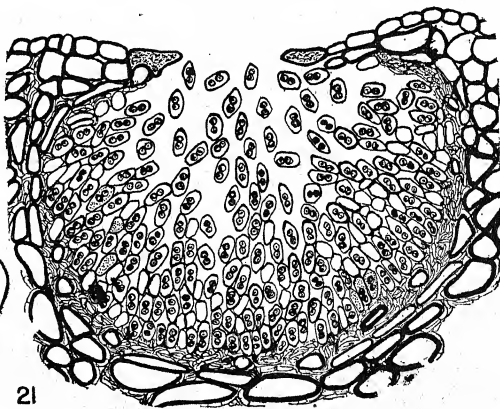
FIGS. 17-22.—Fig. 17, mature teliospores with syncaryon nucleus. Figs. 18, 19, germinating teliospores showing position of pedicel. Fig. 20, plectenchyma replacing pycnosporophores within pycnium. Fig. 21, aecium. Fig. 22, pycnium in margin of aecial cup.

tion grows out into a blunt process. The promycelium grows by the side of the stalk, usually bent at an angle, and its contents develop a slightly orange tint. The first wall is cut off within the teliospore itself, and in most of the cases—as

found that, while the 4-celled promycelium is the rule in *Cystopsora* (fig. 8), a 2-celled promycelium also occurs (fig. 7). Examination of the type material of *Cystopsora oleae* deposited in the Herbarium Cryptogamie Indiae Orientalis
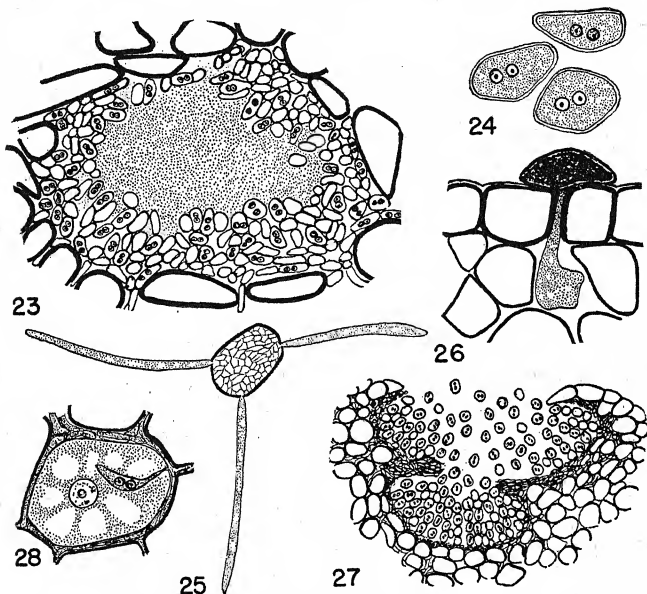
FIGS. 23–28.—Fig. 23, aecial plectenchyma. Fig. 24, aeciospores. Fig. 25, aeciospore germination. Fig. 26, penetration of cuticle by germ tube of aeciospores. Fig. 27, coalescence of superposed aecia. Fig. 28, haustorium within host cell.

the promycelium elongates—it carries along with it the stalk also (fig. 19). In later stages the stalk can be seen attached to the second or the third promycelial cell. BUTLER stated that the 2-celled promycelium is the most distinguishing feature of this rust; but it has now been

revealed that in most of the teliospores in the material collected at Khandala by CHIBBER, and at Belgaum by AJREKAR, the teliospores had germinated by forming a 2-celled promycelium, but a few 4-celled promycelia have now been discovered by careful examination. CHIB-

BER collected in July, 1909; but a second collection made in the same locality in December, 1941, by Rev. H. SANTAPAU had equal numbers of 2-celled and 4-celled promycelia. In the collections made by the writer in Mysore, 4-celled ones have always predominated. The 2-celled promycelial condition is apparently due to the failure of cross-wall formation, but the exact factors which have led to such a failure are not exactly understood. In the closely allied monotypic genus *Zaghouania*, in which the promycelia are as a rule 4-celled, DIETEL (11) reports that 2-celled promycelia also occur when conditions are not very favorable.

CYTOLOGY

Each young teliospore has one pair of conjugate nuclei, but the two nuclei fuse and form a syncaryon in mature spores. As the promycelial initial begins to develop, the syncaryon moves into the promycelium (fig. 3) and undergoes two successive divisions accompanied by wall formation, ultimately resulting in a 4-celled promycelium, each cell of which has a single nucleus (fig. 4). The sporidia are somewhat thick-walled, with orange-colored contents. They do not germinate *in situ*. While each of the promycelial cells is uninucleate, all the sporidia observed had two nuclei (fig. 10). Hundreds of preparations have been examined, and while the exact dividing process of the sporidial nucleus has not been observed, there is no doubt about the binucleate condition of the sporidia. As far as the observations of the writer go, the mycelium bearing the pycnia, aecia, and telia has been found to be binucleate. The uninucleate condition has been observed only in the mature teliospore after the syncaryon has formed in the 4-celled promycelium and in the pycniospores.

There appears to be, therefore, sufficient justification for considering the binucleate condition in the sporidium and subsequently in the mycelium as the diplophase.

Precocious division of the nucleus in the sporidium has been observed by others. In *Puccinia malvacearum* Bert., ASHWORTH (3), ALLEN (2), and SAVILE (23) have shown that the sporidium nucleus divides before germination of the spore; and the same appears to be true, according to SAVILE, in *Uromyces lespedezae-procumbentis* (Schw.) Curt. and *Melampsora bigelowii* Thuemen. SAVILE even found a quadrinucleate sporidium in the former species. The binucleate sporidia of *Puccinia malvacearum* give rise on germination to a uninucleate mycelium, but binucleate cells again appear during the development of the sorus plexus. The sporidia of *Endophyllum sempervivi* (Alb. & Schw.) de Bary are likewise binucleate, and ASHWORTH (4) suggests that, as the nuclei have to pass (while penetrating the host) through a small aperture, it is easier for two smaller nuclei rather than one large nucleus to do so; if in such a process one gets damaged, the other carries on the development. But in these rusts the binucleate hyphae later become uninucleate. This, however, is not the case in *Cystopsora oleae*. Division of the nucleus takes place even before the sporidium has started to germinate, and the persistence of this binucleate condition becomes manifest in the pycnial initials and the hyphae composing them (fig. 16).

Experiments on infection were conducted in order to follow the subsequent history of the nuclei. Young leaves of *Olea dioica* were sprayed with water and dusted with sporidia and the plants placed in a moist chamber. Bits of inoculated leaves were fixed at intervals of

8 hours for 3 days and then sectioned and stained in the usual manner. It was noted that the germ tubes from the sporidia penetrate the host cells through the cuticle and ramify in the intercellular spaces. In early stages they remain multinucleate without the formation of septa, and as many as thirty nuclei have been counted in a hypha at some distance from the tip. As development proceeds, the nuclei become grouped in pairs or fours and are later separated by septa. This type of development has been observed by HIRMER (16) in *Psalliota campestris* (L.) Fries. The pycnial mycelium, while showing binucleate hyphae for the most part, consists of multinucleate cells also. The pycnial initials are subepidermal and concentrated in the intercellular spaces.

A situation more or less akin to this occurs in *Puccinia arenariae* (Schum.) Winter, which was investigated by LINDFORS (20), an account of which is given by JACKSON (17). In this rust the two nuclei fuse in the usual way and the fusion nucleus passes into the promycelium, where it divides, the division being followed by a cross-wall formation between the daughter nuclei. But when the nuclei divide again no cross walls are formed, so that the promycelium is 2-celled, each cell having two nuclei. Later the two nuclei pass into the sporidium, which is throughout binucleate, so that the diplophase is initiated by the failure to form cross walls in the promycelium.

But in both *Puccinia malvacearum* and *P. arenarias*—the forms in which the diplophase is initiated in the sporidium itself—there is no pycnium formation. According to JACKSON (17) the pycnia always develop on the haploid thallus, the pycniospores being uninucleate. They are always present in the eu-forms, whether heteroecious or autoecious; however, they are likely to be absent in opsis forms that appear to be transitional in character. In a great majority of microforms they are stated to be absent, especially in those with a predominantly binucleate mycelium.

The presence of well-developed pycnia on what in fact is a diploid mycelium in *Cystopsora oleae* is therefore a phenomenon at variance with accepted concepts. JACKSON (17) is of the opinion that the presence of pycnia indicates that the rust is heterothallic and that the absence or under-development of pycnia would indicate that the rust is homothallic. BROWN (5), who showed that *Puccinia coronata elaeagni* Fraser & Ledingham produces abortive pycnia occasionally and that *P. grindeliae* Peck produces them only on one of its hosts, holds the same view. Even though monosporidial infection experiments have not been carried out on *Cystopsora oleae*, the initiation of the diplophase in the sporidium indicates that this rust is also homothallic. The pycnium has, since the classical work of CRAIGIE (10), been interpreted as a structure having to do with sexuality in rusts. In homothallic microcyclic forms, JACKSON (18) states that the pycniospores may no longer be functional. That may be the case in *C. oleae*, but the formation of these structures on a diploid mycelium suggests that the sexual function is not a necessary attribute of the pycnium.

In a previous paper, the writer (25) has reported the occurrence of aeciospores and teliospores within the pycnial cups of *Uromyces hobsoni* Vize. According to JACKSON (17), the potentiality is genetically present for any given species for the immediate production of any spore-form which occurs in its life history as soon as the change from the haploid to the diploid phase has taken place,

since such forms are sooner or later pro-
duced by the mycelia descended from
the fusion cells. Occurrence of aecio-
spores and teliospores in the pycnia of
U. hobsoni is thus clear, for the mycelium
had entered the diplophase in the sporidi-
um itself. In *Cystopsora oleae* the forma-
tion of aeciospores in the pycnial cups
has also been noted (figs. 13, 14, 15). In
young pycnia each basal cell possesses
two large nuclei of the expanded type, as
classified by SAVILE (23), and from such
cells aeciospores are abstricted in chains
of two or three into the cavity of the
pycnium. Later, in many cases the basal
cells (other than those that have en-
larged) multiply actively, grow into the
cavity of the pycnium, and gradually dis-
place the pycniosporophores (figs. 14,
20). Various stages in the development
of this plectenchyma have been ob-
served. In some cases only the ostiole and
a few pycniosporophores remained in
the original pycnium, while in others the
entire contents became displaced.

While observing the development of
the aecia, the development of a small
pycnium from the hyphae lining the
margin of the aecium has been noted
(fig. 22). In another instance both the
pycnium and the aecium were seen to
differentiate from the same plecten-
chyma mass, part of the initial having
formed the pycnium and part the aecium
(fig. 9). Such a mode of development is to
be expected, as the mycelium resulting
from the sporidia is already diploid.

In the rusts that have so far been in-
vestigated—such as *Puccinia arenariae*, *P.
anemones-virginianae*, and *P. heuchariae*
—JACKSON (18) states that the diplo-
phase is initiated in the sporidium itself
but the promycelium is 2-celled owing to
the failure to form cross walls. Such a
failure occurs also in *Cystopsora oleae*, for

the collections of rusts made at Khandala
and Belgaum and studied by BUTLER (9)
had a majority of promycelia with two
cells. The rust is apparently in an unsta-
ble condition; and instead of a 4-celled
promycelium, with the consequent neces-
sity for a third division of the nucleus in
the sporidium, it is probably tending to
develop 2-celled promycelia, so that only
two divisions of the syncaryon suffice.
Rather than a case of arrested develop-
ment, the 2-celled promycelium may be
an advance suited to the peculiar nuclear
history of this rust.

The two nuclei in the sporidium which
are evidently conjugate nuclei are held
by JACKSON (18) to be chromosomally
alike. But they should be considered as
having sexually differing potentialities,
and BULLER (7) suggests that such nu-
clei have a mixed sex tendency, basing
this view on the work of HANNA (13, 14),
HARDER (15), and SASS (22).

EXPERIMENTS ON INFECTION

Experiments carried out to confirm
the autoecious nature of the rust, which
had already been established by AJREKAR
(1), would not call for particular mention
but for the fact that further complexities
in the life cycle have been encountered.

For these experiments, test plants
were grown in pots under rust-free con-
ditions. Young leaves were sprayed with
water and then inoculated with fresh
sporidia and the plants placed in moist
chambers. Infection spots became visible
on the fourth day as dark green specks
on the pinkish leaves. With the gradual
enlarging of the spots the infected por-
tions became hypertrophied, resulting in
a malformed condition of the leaves.
Pycnia were seen on the tenth day as
orange-colored specks, and young aecia
became visible on the infection spot 13

days after infection, masses of aeciospores beginning to erupt after rupture of the epidermis.

When aeciospores became available, young leaves of the host plant were sprayed with water, dusted with aeciospores, and placed in a moist chamber for 48 hours. As with the sporidial infection, the spot became visible on the fourth day and spread into a hypertrophied patch. The first spore-form to develop on this malformed structure was —strangely enough—a pycnium, which later became displaced by the aecium. The aeciospores from aecia produced by secondary infection were collected and again used for infecting young leaves of *Olea dioica*. The same symptoms became manifest, and pycnia were again produced as with the first spore-form, these later becoming displaced by the aecia. Such a series of tests with aeciospores of the previous generation was continued up to six generations, with identical results.

It is manifest that the aeciospores of *Cystopsora oleae* have assumed the role of sporidia, in that they develop pycnia on infecting young leaves of *Olea dioica*. The aeciospores never germinate by the formation of sporidia as in some of the microcyclic rusts that have been investigated. In *Endophyllum sempervivi*, for instance, the aeciospores always germinate by the formation of a promycelium with basidiospores and are usually termed telioid aeciospores. In *Kunkelia nitens* (Schw.) Arth., DODGE (12) pointed out that normal aeciospores and telioid aeciospores are associated within the same sorus. In *Gymnoconia interstitialis* Lagerh., on the other hand, where normal two-celled teliospores also occur, KUNKEL (19) pointed out that the aeciospores in certain seasons germinate by

the formation of a promycelium with basidiospores.

Recently, BROWN (6) has shown that when the urediospores of *Puccinia minussensis* Thuemen are sown on seedlings of *Lactuca pulchella*, secondary uredia develop in abundance but the infection later becomes gradually systemic and spreads toward the base, into the rhizome. When these rhizomes are removed and replanted in pots, the emerging shoots are studded with pycnia. BROWN has shown, however, that a de-diploidization of the diploid mycelium takes place, resulting in the formation of haploid pycnia and haploid protoaecia. The same phenomenon has been demonstrated in *Puccinia suaveolens* (Pers.) Rostrup by BULLER and BROWN (8). BULLER therefore concludes that in rust fungi with systemic mycelia that perennate in the subterranean organs of herbaceous hosts, de-diploidization of the diploid mycelium takes place annually.

Evidence of such de-diploidization has not been seen in *Cystopsora oleae*, for even the pycnial initials are binucleate, and a haploid mycelium has not—as already stated—been observed in the numerous preparations examined.

The experiments on infection have been carried out at different times of the year, using aeciospores, and it is beyond doubt that young leaves, irrespective of the season, invariably develop pycnia as the first spore-form, followed by aecia. It was thought that telia which do occur on the host plant in nature are formed by the infection of mature leaves. Experiments on a large scale were therefore undertaken, and mature leaves of *Olea dioica* were sprayed with water and dusted with aeciospores on the undersurface. The inoculated plants were placed in moist chambers for 48 hours and later re-

moved to a greenhouse for observation. There were no visible signs of infection for 65 days, but yellow specks began to appear on the undersurface on the sixty-sixth day, gradually developing into circular orange-yellow spots. Emergence of the sporogenous tissue and development of the telia and teliospores were observed after eighty days. Repeated experiments with mature leaves in the manner just described have given confirmatory results.

It is manifest that the spore-forms which develop as a result of aeciospore infection are determined by the maturity of the leaves. On younger leaves the aeciospores assume the role of sporidia, without forming a promycelium, however, and later assume the role of normal aeciospores on older, mature leaves. To determine the stage at which the aeciospores produce telia, a small shoot on a potted plant was selected for experiment. Leaves developing in acropetal succession were infected with aeciospores in the usual manner and the plant kept in a moist chamber. The youngest leaves at the top formed infection spots in 6 days. These became hypertrophied, and pycnia and aecia were formed in due course. The oldest leaves at the base showed no signs of infection, even on the fiftieth day, but the leaves midway showed an interesting type of development. Some of them developed swollen infection spots with pycnia and aecia, but at places where no malformations had taken place, telia were observed after 30 days. On older leaves, however, telial formation took much more time; in fact, sometimes more than 100 days elapsed before the telia were manifest. The maturity of the host tissue therefore determined not only the time taken for the development of telia but also the nature of the spore-form that developed.

Diagnosis of genus

The morphological facts now brought to light have necessitated an emendment of the generic and specific descriptions.

Cystopsora Butler, emend

Pycnia subepidermal, flask-shaped. Aecia subepidermal, cupulate, without peridia. Telia subepidermal, sporogenous hyphae emerging through stomata in fascicles; hyphae quite distinct, without lateral coalescence; teliospores developed in clusters from basal generative cell, formed at apex of sporogenous hypha; teliospores germinating without a rest period and directed toward the host; promycelium semi-internal, 2- or 4-celled; sporidia sessile; teliospores without germ pores.

Type species: *Cystopsora oleae* Butler on *Olea dioica* Roxb. at Khandala and Belgaum.

Cystopsora oleae Butler, emend

Pycnia amphigenous, distributed on hypertrophied patches and "witches' brooms," orange-yellow, flask-shaped, with ostiolar filaments. Aecia formed by displacing pycnia on hypertrophied patches, cupulate, yellow, erumpent; aeciospore chains converging toward center, peridia absent; aeciospores polyhedral, yellow, ovate-ellipsoid to fusiform, with reticulations on exospore and three distinct germ pores; aeciospores often developing within pycnia. Telia hypophyllous, sparsely distributed, orange-yellow, pulverulent, turning whitish following germination of teliospores, not causing hypertrophy on infection patches; sporogenous hyphae exserted through stomata in fascicles, distinctly separate; teliospores developed in clusters on discoid basal cell, 1-celled, pedicellate, spherical or ovate, deep orange-yellow, minutely verruculose; germina-

tion at maturity without a rest period, within sorus; promycelia directed toward the host, 2- or 4-celled, semi-internal; sporidia rather thick-walled, oval, sessile, binucleate.

Habitat on leaves and twigs of *Olea dioica* Roxb. at Khandala, Belgaum, Balehonnur (Mysore), and Bannerghatta (Bangalore).

Summary

1. *Cystopsora oleae* has both 2- and 4-celled promycelia. The rust is an autoecious opsis form and causes hypertrophy and "witches' brooms" on young leaves and twigs. Infection patches on which telia are formed, however, are not malformed.

2. Teliospores germinate *in situ* without a rest period. Young teliospores are binucleate. Fusion takes place in mature spores. Promycelium is formed by the extension of the lower part of the teliospore, and the syncaryon moves into it and divides twice. If the promycelium is 4-celled the cells are uninucleate but are binucleate if it is 2-celled. The sporidia are always binucleate.

3. The binucleate condition persists, so far as it has been possible to determine, and it is presumed that the diplophase is initiated within the sporidium itself. The pycnium is thus borne on a diploid thallus, as all the associated mycelium has a pair of conjugate nuclei in each cell, although the pycniospores are uninucleate. Formation of aeciospores within the pycnia has been observed many times.

4. When aeciospores are sown on young leaves, they invariably give rise to pycnia, although they have never been observed to form promycelia and sporidia. When older leaves are infected, teliospore development takes place, but after a lapse of several weeks.

5. This rust thus presents certain remarkable characteristics not previously observed among other rusts and appears to be a form in a very unstable condition.

6. The diagnostic characters of the genus and species have been emended.

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TRANSLOCATION OF THE REPRODUCTIVE
STIMULUS IN SUGAR BEETS

MYRON STOUT

Introduction

Sugar beets vary widely in their ther-
mal requirements for reproductive de-
velopment. Most of the economic varie-
ties require prolonged exposure to cool
temperatures (that is, extensive thermal
induction) before they will produce
seedstalks. They may be regarded as
biennials. This general characteristic is
due principally to the fact that generally
in sugar-beet breeding the effort has
been made to suppress the bolting tend-
ency, since production of seedstalks is
undesirable. There are non-economic
varieties that require so little thermal in-
duction that they will bolt, even when
planted during the hot summer months
in the vicinity of Salt Lake City, Utah.
Such varieties may be classed as annuals.

They have been found useful in certain
types of experimental work.

All beet varieties, however, require
long photoperiods before they will bolt
normally. And all varieties may be kept
in a vegetative condition indefinitely by
growing them in an environment of short
photoperiods. and warm temperature.
Furthermore, even though reproductive
development has been initiated in an en-
vironment favorable to bolting, rever-
sion to the vegetative type of growth
may be effected by moving the plants to
an environment of short photoperiods
and warm temperature.

Annual beets that have started to bolt
and then reverted to a vegetative condi-
tion have rosettes of leaves at the ends
of the seedstalks. These terminal areas

react to photoperiods in the same way as do annual beets that have not bolted previously. Use of this fact has been made in some experiments here reported to obtain annual beets with growing points widely separated and hence more easily subjected to differences in light exposures.

The fact that the response of plants to photoperiod is localized rather than systemic was shown by GARNER and ALLARD (8) in 1925. Other workers have shown that the photoperiodic stimulus can be translocated to a part of the plant serving as receptor by defoliating this part and thereby inducing a flow of food into it.

The work reported here is mainly based on the photoperiodic response of beets; but the fact is recognized that, at least with biennial beets, thermal induction is an important factor. These two factors exert their influence on separate parts of the plant. KNOTT (12) and others have shown with various plants that the photoperiodic sensitivity is localized in the leaves. Unpublished studies by the writer have shown this to be the case in annual beets. CURTIS and CHANG (7) and CHROBOCZEK (6), working with celery and beets, respectively, showed that the thermal induction stimulus is perceived in the crown. The evidence that light and temperature are complementary factors encouraged the effort to conduct the present studies with the light factor alone.

Grafting as a means of studying photoperiodic response of plants has been employed by a number of workers (3, 4, 10, 11, 13, 14, 15, 17). CHOLODNY (5) has reviewed several of these papers. It has been shown that the substance or stimulus produced in a short-day plant as the result of exposure to short photoperiods is similar to that produced in a long-day plant by long photoperiods. MELCHERS (13) induced flowering in biennial plants of *Hyoscyamus niger* L. by grafting on them flowering scions of annual plants or of low-temperature treated biennials. NAYLOR (15) grafted annual and biennial varieties of sugar beets and grew both parts under long photoperiods, but since he did not provide the conditions necessary for translocation of food between the grafted parts, each section developed independently of the other. The annual part bolted while the biennial part remained vegetative.

The use of annual-biennial grafted sugar beets was thought to afford an excellent means for studying certain questions that naturally arise in considering the thermal and photoperiodic factors in reproductive development in relation to the kind of substance or stimulus produced by each. Do annuals require only one kind of substance or stimulus, while biennials require two kinds? Or, do both of these environmental factors contribute toward the production of one and the same kind of substance or stimulus? Further information concerning the answers to these questions should aid in the solution of the internal causes of reproductive development. Considerable information has accumulated concerning the environmental factors responsible for reproductive development in plants, but as yet little or nothing is known about the mechanism of the stimulus or the substance or substances involved.

Material and methods

A variety of annual beets, usually referred to as the Munerati annual, S.L. 2240 or S.L. 841, was used in the early translocation studies. In later studies with grafted beets the annual component was a hybrid between the Munerati annual and a curly-top-resistant biennial variety. This hybrid, S.L. 2850, was

found to be definitely annual in character and had the advantage of better vigor and some curly-top resistance. S.L. 234, a nonbolting curly-top-resistant variety, was used as the biennial
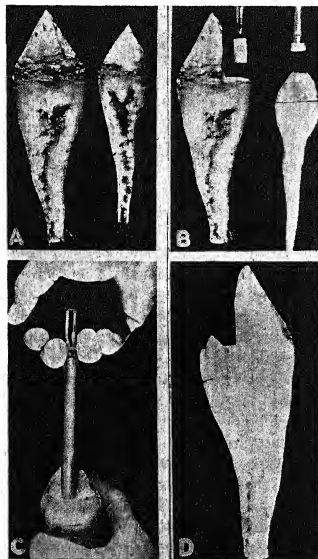
Fig. 1.—Corkborer technique used to graft sugar beets. A, beet used as stock at left, that used as source of scion at right. B, cylinders of tissue removed from both stock and scion. C, heavy-walled metal tubing, reamed out to fit conical shoulder of scion, used to force scion from corkborer into stock. D, longisection of grafted beet.

component in all the grafting experiments.

Annual beets having more than one growing point were obtained by two methods, both of which gave plants that reacted similarly. In the first method the leaves and petioles of vegetatively growing plants were trimmed off and the tops split to a point about 2 inches below the crown. A pebble was placed in the crotch and the beets were potted and grown in a warm, short-photoperiod environment for about 2 months, or until a single well-developed rosette of leaves was obtained on each segment. In the second method the plants were allowed to start to form seedstalks, the tops of the seedstalks were pinched off to induce lateral branching, then the beets were placed in a warm, short-photoperiod environment until single, well-developed rosettes of leaves were formed on two or more branches. The latter method required more time but produced plants that were more easily covered.

Heavy black cloth bags covered with white sheeting were used to cover parts of the plants to be kept darkened. The bags were kept from collapsing by inside wire frames and were closed with drawstrings. The exposed parts were given 17- to 18-hour photoperiods or continuous illumination. Supplemental illumination intensity was 25–50 foot-candles. In preliminary experiments it was found that the tips of seedstalks growing in the dark turned black and died after several weeks' treatment. An exposure of the covered part of the plant to about 2 hours of sunlight each week prevented this trouble. In all subsequent tests the plants were given this prophylactic treatment.

GRAFTING TECHNIQUE.—Several attempts were made to graft beets so that the scion would become entirely dependent upon the stock for mineral nutrients. The only method that proved satisfactory was based on one described by Goss (9) for inoculating potatoes with spindle-tuber disease. Use of that method for beet work was suggested to the writer by C. W. BENNETT, who had used it in curly-top studies at Riverside, California.

As used in the present work, the method is illustrated in figure 1 and may be described as follows: A beet about $1\frac{1}{2}$–$2\frac{1}{2}$ inches in diameter was used for the stock. The leaves were trimmed closely and a block of the crown tissue cut away to make a flat shoulder on one side, at about the level of the lowest leaf scar. From this shoulder, and extending down to a parallel incision about $\frac{3}{4}$ inches below, a cylinder of beet tissue about 11 mm. in diameter was removed by means of a corkborer.

A smaller beet was used for the scion. It was trimmed to remove the leaves and some of the crown tissue but without injury to the apical bud. A transverse incision was made in the shoulder of the beet and well past the center of the crown. This incision was at such distance from the top of the crown as to make the cylindrical scion the desired length to fit the space in the stock beet. The scion cylinder, including the apical bud, was cut by a corkborer about 1 mm. larger in diameter than the one used to make the space in the stock.

The scion was then forced from the corkborer into the stock by means of a heavy-walled, metal tube slightly smaller in diameter than the corkborer. The end of this tube was reamed out to fit the conical top of the scion, permitting pushing the scion without excessive injury. The scion was so inserted that the upper edge of its cylindrical surface was flush with or slightly above the flat surface of the stock. The cut surfaces, with the exception of the apices of the scion and stock, were then covered with melted grafting wax. Beets so grafted were kept in a humid chamber for about 2 weeks before they were transplanted to pots.

The grafted beets were grown in a warm, short-photoperiod environment for several months to reverse any ther-mal induction they had received. It was found that if the beets were taken from the field in the fall, when the crown tissue was firm, 75–90% of the scions grafted by this method grew. The crown tissue of greenhouse-grown beets was not firm enough to work well with the method.

Experimental results

ANNUAL BEETS

Seven split annual beets were placed under differential conditions of illumination February 3, 1938. One shoot of each plant was exposed to 17-hour photoperiods; the opposite shoots of three plants were kept in continuous darkness; while the opposite shoots of the other four plants were exposed to 7-hour photoperiods. From 3 to 4 weeks were required to induce bolting in the 17-hour exposed shoots. Of the three that had one shoot kept in darkness, one darkened shoot bolted March 10, another April 1, and the third died before April 1. Of the four plants that had shoots exposed to 7-hour photoperiods, one died and the other three remained vegetative.

In another test, one shoot of each of six split annual beets was exposed to 17-hour photoperiods, beginning April 4, 1938. The opposite shoots of three plants were kept in continuous darkness, while the opposite shoots of the other three plants were exposed to 4-hour photoperiods. The 17-hour exposed shoots bolted about April 25. The continuously covered shoots developed as follows: one bolted May 25, another June 6, and the third developed an elongated crown but did not form a typical seedstalk; all the three shoots exposed to 4-hour photoperiods remained vegetative.

In the previously described tests with split annual beets, a period of about 3–4

weeks was required to induce bolting in the long-day exposed part and about a month of additional time to induce bolt-

FIG. 2.—Annual beet with three shoots that were exposed to photoperiods, after May 28, 1941, as follows: left, 7 hours; center, continuous darkness; right, continuous illumination. Shoot at right bolted June 5; one in center had seedstalk measuring 3 inches June 18; one at left remained vegetative. Photographed August 9, 1941.

ing in the attached part kept in the dark. In tests with beets that had bolted and then reverted to a vegetative condition, the time required to induce bolting in

continuously exposed parts was only about 2 weeks, and bolting of the part kept in the dark followed that of the continuously illuminated part much more closely. In some instances the part exposed to continuous illumination and that kept in continuous darkness bolted almost simultaneously. Only one of the latter tests will be described in detail.

Seven annual plants that had bolted and then reverted to a vegetative condition were given differential photoperiodic treatments beginning May 28, 1941. Two plants had three shoots each, and each shoot was given a different treatment: continuous illumination, 7-hour photoperiods, and continuous darkness. Four plants having two shoots each had one shoot exposed to continuous illumination and the other to continuous darkness. The other plant, having two shoots, had one exposed to continuous illumination and the other to 7-hour photoperiods. In all, there were seven shoots exposed to continuous illumination, three to 7-hour photoperiods, and six to continuous darkness. Seedstalks had started to develop on all shoots given continuous illumination by June 5. All those kept in continuous darkness had seedstalks measuring $1\frac{1}{2}$–$5\frac{1}{2}$ inches in length by June 18, indicating that bolting had started in some about a week earlier. All the growing points exposed to 7-hour photoperiods remained vegetative. The typical effect of each treatment can be illustrated in one of the plants that had three shoots, each treated differently. The plant shown in figure 2 indicates that the reproductive stimulus translocated from the continuously illuminated part (right) to that kept in darkness (center) was not appreciably decreased or inhibited by any negative stimulus from the attached vegetative part that received 7-hour photoperiods (left).

ANNUAL-BIENNIAL GRAFTED BEETS

Annual (variety S.L. 2850) and biennial (variety S.L. 234) beets were taken from an overwintering seed-field in St. George, Utah, March 10, 1943. They were grafted March 10 and kept in a warm, humid chamber for about 2 weeks and then potted and grown in a warm, short-photoperiod environment (to reverse thermal induction [16] in the biennial variety) until August 13, 1943. During this period the beets were frequently trimmed of all but one vegetative shoot on each stock or scion. After August 13, one part of each plant was given continuous illumination and the other part kept in darkness.

Seven plants, in which the annual stock was kept in darkness, were started in the test. Four of the plants died. The three that lived are shown in figure 3A. Both parts of each plant remained vegetative, with one exception. The annual part of the large plant at the left started seedstalk formation early in the test (September 27), but subsequent elongation of the seedstalk did not follow, and when the photograph was taken (December 13) the growth had reverted to a vegetative condition. It is probable that small leaflets that developed on the crown of the annual part below the cover were not removed frequently enough at first and may have been responsible for initiation of the seedstalk, rather than any stimulus translocated from the exposed biennial part. The fact that the seedstalk reverted to a vegetative rosette after being initiated further supports this explanation.

Eight grafted plants, in which the annual scion was kept in darkness, are shown in figure 3B. With one exception, both parts of each plant remained vegetative throughout the test. The plant at the upper left of figure 3B grew vegetatively until November 19 (98 days), when a seedstalk started to develop on the exposed biennial stock. The cover on the annual scion was removed and a seedstalk had also started to develop there. This was the only instance in which a light-exposed biennial part of

FIG. 3.—Annual-biennial grafted beets in which biennial part (left side of each beet) was exposed to continuous illumination while annual part was kept in darkness. A, biennial part as scion; B, biennial part as stock. Differential lighting started August 13; photographed December 13, 1943.

the grafted plants bolted, and it was probably caused by cooler temperatures of the greenhouse during the last part of October and the month of November. The fact that only one of the biennial beets in this group of plants bolted is probably due to genetic variation.

Four annual-biennial grafted beets, in which the biennial scion was kept in continuous darkness, are shown in figure 4A. The continuously illuminated annual stocks had all started to bolt by Septem-

ber 13. The two biennial scions on the left of the figure bolted, one on October 5 and the other on December 8. Lack of vigor of the other two biennial scions may have been responsible for their remaining vegetative.

bolted (from left to right) September 27, October 5, and September 27. Three to six weeks' exposure to continuous illumination was evidently required to produce the stimulus or substance in the leaves of the annual, translocate it to the apical
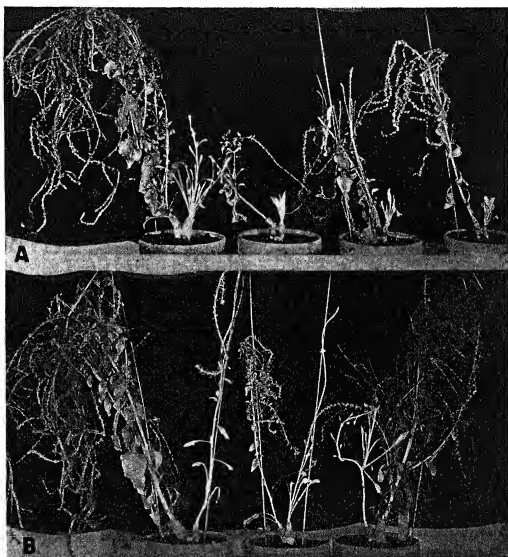
* FIG. 4.—Annual-biennial grafted beets in which annual part was exposed to continuous illumination while biennial part was kept in darkness. A, annual part as stock; B, annual part as scion. Differential lighting started August 13; photographed December 13; 1943.

Three grafted plants, in which the biennial stock was kept in continuous darkness, are shown in figure 4B. The continuously illuminated annual scions bolted on the following dates: From left to right, September 6, 27, and 13. The continuously darkened biennial stocks

bud, and induce formation of a seed-stalk. It is also evident that only 8–21 days of additional time was necessary to induce bolting in the continuously darkened biennial parts of the same grafted plants. This evidence of effective similarity regarding requirements for reproduc-

tive development in annual and biennial beets will be discussed more fully later.

Discussion

Production of a food deficit in one part of a plant by defoliation or by keeping it in darkness has proved a useful tool in the study of the translocation of substances other than plant food. The work of BENNETT (1) on the translocation of viruses showed that curly-top virus was rapidly translocated from a diseased to a healthy shoot of a sugar beet when the healthy shoot was defoliated or kept in darkness. In some instances 24 hours was sufficient to translocate the virus to the healthy shoot. In this case a short period of translocation would supply the small amount of virus necessary to initiate the disease. On the other hand, to cause bolting, a continuous translocation of reproductive stimulus or substance probably is required.

When annual beets were split similarly to those used by BENNETT, 3–5 weeks of darkness were required for evidence of bolting in the darkened side after the illuminated side had started to bolt. In these instances the two growing points were separated by a considerable reservoir of storage tissue, upon which the darkened shoot probably drew for food as well as from the shoot exposed to long photoperiods. Because the food for the darkened shoot presumably came from these two sources, accumulation in this shoot of an adequate amount of the reproductive substance was delayed. There was only about half as much lag in bolting response between the exposed and the darkened shoots in the beets that had bolted and then reverted to the vegetative state before being given the differential illumination. The growing points in these beets, even though farther apart than in the split beets, were separated by

stem tissue rather than by storage tissue. The difference in time required between the two types of plants to develop seedstalks is probably further evidence that a synthesized, flower-inducing substance, translocated with the elaborated food, is involved in reproductive development. The negative results of NAYLOR (15), who grafted annual and biennial varieties of sugar beets, also show that translocation of the reproductive substance follows that of synthesized food. Because he created no food deficit in the biennial, there was no transfer of reproductive substance from the annual part.

The darkened shoot of annual beets that had three shoots, one of which was kept vegetative (fig. 2), bolted as rapidly as darkened shoots on plants that had no vegetative shoots, indicating that vegetatively growing plants do not produce any substance or stimulus antagonistic to reproductive development but merely lack the substance or stimulus in sufficient amounts to cause it. This evidence confirms the conclusions of CAJLACHJAN (2) in this regard.

Several workers have shown that the substance or stimulus produced in long-day plants under long photoperiods is in effect the same as that produced in short-day plants under short photoperiods. They used defoliation of the receptor to induce translocation of the flowering stimulus. MELCHERS (13) showed that biennial plants of *Hyoscyamus niger* L. could be forced to initiate reproductive development by grafting to them either flowering scions of annuals or scions from cold-treated biennial plants. He postulated that the substance produced by the annuals and biennials was the same, but that a gene in the biennial introduces an inhibitory effect that is eliminated by treatment at low temperature. The available data indicate that the

flower-inducing substance or stimulus
produced in annual beets under the in-
fluence of long photoperiods alone is in
effect similar to that produced in biennial
beets as a result of the combined effect of
cool-temperature exposure and long pho-
toperiods.

If the flower-inducing substance is the
same in annual and biennial beets, it
would appear that the leaves of the an-
nual can synthesize an adequate amount
of the substance under long photoperiods
and that the leaves of the biennial can-
not, except possibly under continuous
illumination of high intensity. Another
possibility is that an activator or precur-
sor is genetically supplied to the annual,
while in the biennial it is formed as a re-
sult of cool-temperature exposure.

The fact that the two environmental
factors, light and temperature, affecting
respectively the leaves and the crown,
produce a similar effect suggests the pos-
sibility of obtaining evidence on the bio-
chemical basis of reproductive develop-
ment. Possibly the same flower-inducing
substance will be discovered to be formed
in beet leaves and crowns.

Summary

1. Separate shoots of annual beets
having more than one shoot were sub-
jected to differential photoperiods as fol-
lows: long days or continuous illumina-
tion, short days, and continuous dark-
ness. The parts exposed to long days or
continuous illumination developed seed-
stalks. The parts exposed to short days,
even though connected to parts exposed
to long days or continuous illumination,
remained vegetative. The parts kept in
continuous darkness and connected to
parts exposed to long days or continuous
illumination developed seedstalks, even
when they were also connected to short-

day exposed parts that remained vegeta-
tive.

2. These results indicate that sub-
stances conducive to reproductive devel-
opment may be translocated with the
carbohydrates. Exposure of a part to
short photoperiods produced sufficient
carbohydrates for that part, and hence
none was translocated to it from the
long-day part. Keeping a part in con-
tinuous darkness made that part de-
pendent on the long-day exposed part,
and hence food, and with it reproduc-
tive-inducing substances, were translo-
cated to the darkened part.

3. Continuously illuminated biennial
parts of annual-biennial grafted beets
failed to develop seedstalks when they
were not also subjected to cool-tempera-
ture exposure. Neither did the continu-
ously darkened annual parts of the same
beets develop seedstalks.

4. About one month was required to
initiate seedstalks in continuously illumi-
nated annual parts of annual-biennial
grafted beets. The continuously dark-
ened biennial parts of most of the same
beets also developed seedstalks during
the following month.

5. These results indicate that the bien-
nial is not able to synthesize reproduc-
tive-inducing substances as a result of
photoperiod alone, as annuals do, but
also requires cool-temperature exposure.
The reproductive-inducing substances
produced by biennials as a result of ther-
mal and photoperiodic induction, and
those produced in annuals as a result of
photoperiodic induction alone, appear
to be in effect similar.

6. A simple and effective method of
grafting such material is described.

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CICATRIZATION IN LEAVES OF BRYOPHYLLUM CALYCINUM

WALTER B. WELCH

Introduction

The leaves of *Bryophyllum calycinum* Salish. are well known for their ability to retain moisture after having been detached from the plant. During an investigation on the water relations of leaves during severe drying (7), small corky layers were found to have formed over the end of the petiole when the leaves were allowed to dry in the open air. These same corky layers were seen at any injured place on the leaf. When the leaves were transferred from their drying trays to the pan of a balance for weighing,

the corky layers would drop from the leaf.

SAMUEL (5) states that in a moist atmosphere with a good supply of water to the leaf all injured tissues will be abscised and that in a dry atmosphere with a poor supply of water to the roots abcission will not occur. He states further that in a moderately dry atmosphere abscission will or will not occur, depending upon whether the meristem arises some distance within the leaf lamina or near the edge of the injury. PRIESTLY and WOFFENDEN (4) found in potato tubers that,

if the injured tubers were exposed to moist atmosphere, the healing of wounds was more rapid and continuous; and if exposed to dry atmosphere, especially in sunlight, the healing was slower and not

FIG. 1.—Outline of leaf of *Bryophyllum calycinum* showing positions of wounds. *A*, wound along margin of leaf; *B*, cross-hatch wound of center; *C*, wound of petiole.

continuous. BLACKMAN and MATTHAEI (1) found that "the factor of excessive drying does not come in."

Since the corky layers did fall from the leaf with the appearance of true abscission, an investigation was undertaken to

determine whether there was an active meristem and abscission layer of the cicatrice formed in the leaf. WYLIE (8) has found that the thicker the leaf the wider the cicatrice, except in succulents or semi-succulents. There seems to be no constant relation for all leaves. The leaves of *Bryophyllum* might certainly be called succulent, or they might be considered xerophytic—if MAXIMOV's (2) criteria were used. The leaves of *Bryophyllum* will produce small plantlets on the margin at the notches, even after the leaf has wilted, if the leaf is removed from the plant in a turgid condition. If the leaf is allowed to wilt as the plant dries up and is then removed, it will produce few if any plantlets.

Material and methods

The material of *Bryophyllum calycinum* used in this investigation was that which is known as the Chicago variety (3). The plants were grown in the greenhouse at the University of Chicago, and also in the greenhouse of Southern Illinois Normal University, under ordinary greenhouse conditions until they had produced at least eight sets of leaves. At the fourth and fifth nodes the leaves no longer increase in size. These were considered mature and used in this investigation.

Two series of leaves were established. In the first the leaves were detached from the fourth and fifth nodes of vigorously growing plants. These were placed on screen-wire trays and allowed to dry at room temperature varying from $50°$ F. to $85°$ F. and at laboratory humidity from 50% to 85%. In the other series the leaves remained attached to the plants that were growing under greenhouse conditions. The temperature of the greenhouse was never over $85°$ F. or under $55°$ F. The relative humidity was

rarely as much as 90% and never under 60%.

The series of leaves allowed to dry in the laboratory was divided into three sets. One set was cut along the margin with a sharp razor blade (fig. 1A; hereafter shown as A in all figures). This cut was made through all the tissues and the margin removed and discarded. Another set of leaves was cut with crosshatch lines (fig. 1B; hereafter shown as B in all figures). This was about halfway from the midrib to the margin and at about the widest portion of the leaf. These leaves were cut through the upper epidermis and into the mesophyll but not through the lower epidermis. The third set had the petiole cut off about halfway from the point of attachment to the base of the blade (fig. 1C; hereafter shown as C in figures).

Leaves of the second series were left attached to the plant and only those from the fourth and fifth nodes were wounded. The wounds were made as in the first series, except at the petiole. It was impossible to make a cut more than halfway across this organ and have it remain on the plant, and it was found more practical to cut through about two-fifths of the tissues, including one of the lateral bundles.

In each series all leaves were wounded on the same day and at as nearly the same time as possible. The first collection was made at once, and then every 4 hours for the next 48 hours. After that, collections were made every day for 2 weeks. After 2 weeks a collection was made every 4 days for the next 9 weeks.

The specimens were fixed in a solution of 6.5 cc. formalin, 2.5 cc. glacial acetic acid, and 50% alcohol to make 100 cc.; mounted in paraffin and sectioned at 10 μ. The drawings were made with the aid of a Promi projector and all were magnified twenty-eight times. In the sections of the leaf blade no attempt was made to include or exclude either the veins or the stomata.

Discussion

The leaf of *Bryophyllum calycinum* has some of the characteristics of a succulent, in that the ratio of internally to externally exposed surface is comparatively low (6). The relative number of stomata is also small (7). In the illustrations the intercellular spaces are indicated only by the curvature of the cell walls as they jut into the intercellular space. In this leaf there is little to distinguish the types of mesophyll cells. After the leaf has started to dry out, the palisade and spongy cells are almost indistinguishable. The cuticle is not as thick as might be expected on a succulent leaf. In the drawings it may even be exaggerated, owing to the width of the line on the surface of the epidermis. There was no attempt to show the contents in any of the cells.

When the first samples were taken they were plunged immediately into the fixative. A little collapse will be noticed in the leaf section taken from the margin A (fig. 2). There is little difference between the leaf that was attached to the plant (fig. 2) and the one that was removed to dry in the open air (fig. 3). The sections taken from the center of the leaf (figs. 4, 5) show more collapse. This may have been due to the razor blade not being very sharp. In the sections of the petiole (figs. 6, 7) where the tissues were more rigid the cut was clean and no collapse was seen. The lack of differentiation in the mesophyll is shown in the drawings. These would be considered the "normal" sections. The wounds were made a matter of minutes before being plunged into the killing fluid and there-
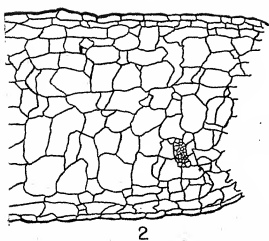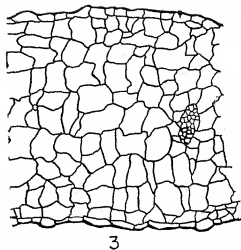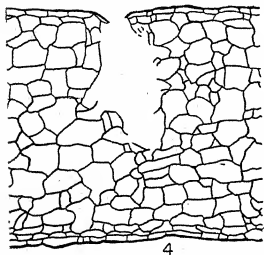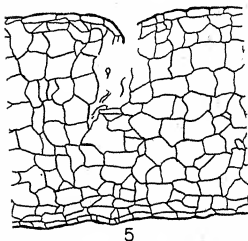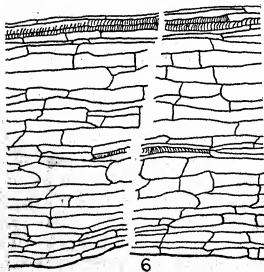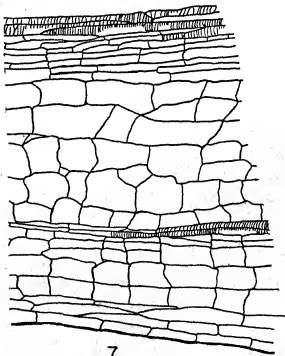
FIGS. 2–7.—Sections of leaves fixed immediately after wound was made, or at 0 hours: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 2, attached leaf. Fig. 3, removed leaf. Fig. 4, attached leaf. Fig. 5, removed leaf. Fig. 6, attached leaf. Fig. 7, removed leaf.

fore the response is none or very little. There is only slight evidence of any destruction of the cells other than that done by the razor.

Four hours after the wounds were made, some changes were evident in the cells along the margin of the wound A. In the sections A (figs. 8, 9) the epidermis curls over the wound, and in some cases the upper epidermis is almost touching the lower. A much greater area has started to dry out in the leaf that was attached to the plant (fig. 8) than in the leaf that was removed (fig. 9). In the attached leaf there are some twelve rows of cells that have started to collapse, while in the leaf that was removed there are only eight rows. The curling of the epidermis and the collapse of the cells adjacent to the wound may be what PRIESTLY and WOFFENDEN (4) refer to as an effective block to the transpiration from a wound surface.

Sections B (figs. 10, 11) show much less of the collapse due to the drying of the cells than sections A. Here the wound was through the upper epidermis and the upper part of the mesophyll. The evaporation from this wound must have been reduced to a minimum, for very little moisture was lost through it. Here also the area of collapse seems to be greater in the leaf that was attached (fig. 10) than in the one that was removed (fig. 11). The difference in the sizes of the leaf is not due to one drying out more than the other. The leaves did vary a great deal in thickness.

In the sections of the petiole at C (figs. 12, 13) there is very little change in the leaf that was attached as compared with the section that was made 4 hours before. In this section the wound was made only two-fifths of the way across the petiole, so that it could remain on the plant (fig. 12). Very little evapora-

tion has taken place, since little of the wound area was exposed. In the section where the end was cut off the petiole (fig. 13) the whole area was exposed and therefore evaporation was great. Almost as much evaporation takes place as in the sections A. The epidermis starts to curl over the cut tissues, and the only difference from sections A is the vascular bundle that keeps the organ rigid.

After 3 days many of the cells that showed collapse in the first 4 hours are dead, and so decomposed as to be unrecognizable. This layer acts as a further block to the evaporation from the living cells below. In the sections A (figs. 14, 15) these areas are shown by the heavy shading. In both figures the cells next to the dead decomposed tissues seem to be as turgid as any others of the leaf. It is apparent that the leaf that was attached (fig. 14) lost more tissue by collapse owing to drying than did the leaf that had been removed. Again it might be pointed out that the leaves that were removed from the plant had no contact with moisture other than that of the air.

In the sections of the cross-hatch wound in the center of the leaf (B), the leaf that was attached (fig. 16) shows much greater collapse than the one that was removed (fig. 17). Relatively there are a greater number of dead cells in the leaf that was removed than in the one that was attached. Here also the living cells next to the dead tissue seem to be as turgid as any of the others.

In the sections of the petiole at C, the leaf removed from the plant (fig. 19) shows a much larger area of the dead decomposed cells than does the leaf that remained on the plant (fig. 18). The leaf that was removed also shows a curling of the epidermis that will partly cover the decomposed cells. This follows the expected procedure, since there was a large
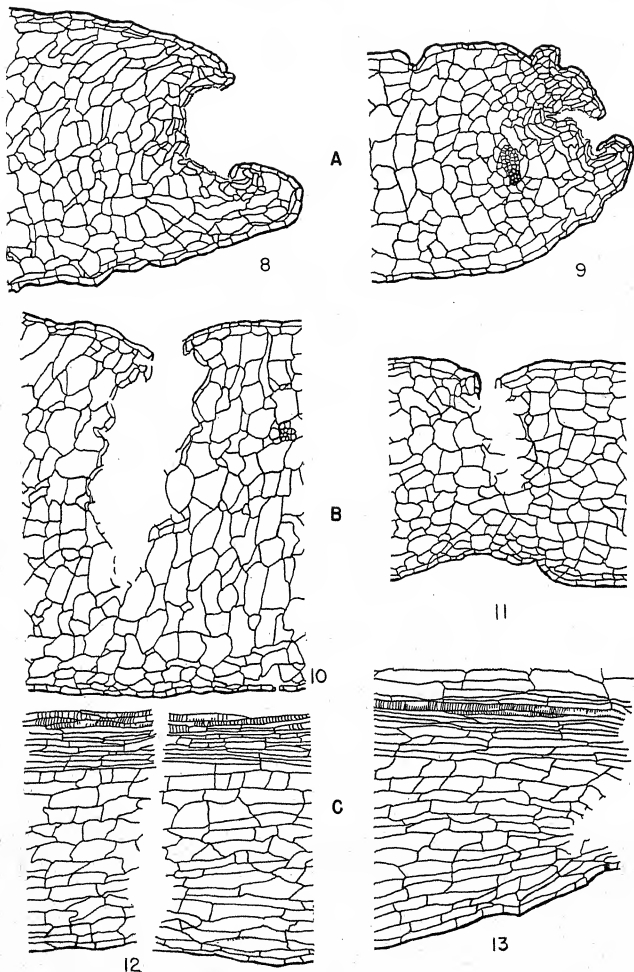
FIGS. 8–13.—Sections of leaves 4 hours after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 8, attached leaf. Fig. 9, removed leaf. Fig. 10, attached leaf. Fig. 11 removed leaf. Fig. 12, attached leaf. Fig. 13, removed leaf.
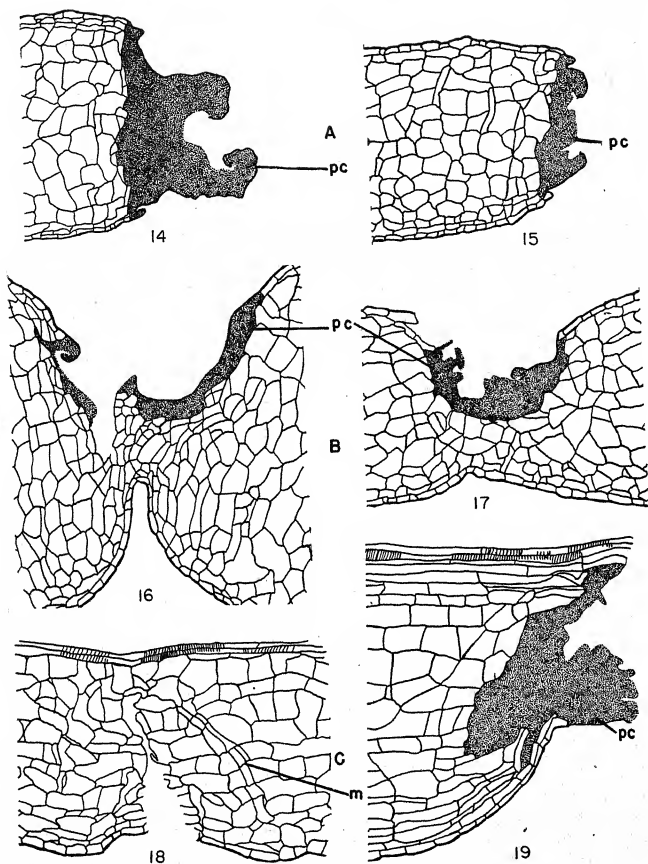
FIGS. 14–19.—Sections of leaves 3 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 14, attached leaf. Fig. 15, removed leaf. Fig. 16, attached leaf. Fig. 17, removed leaf. Fig. 18, attached leaf. Fig. 19, removed leaf. *m*, meristem; *pc*, pseudocicatrice.

area of collapsed tissue in the petiole at 4 hours. There is no decomposition of the cells in the petiole of the leaf that was attached to the plant, and there is very little collapse of the tissues along the wound, but a new structure is developing the meristem that will later form the cicatrice (fig. 18m). In following WYLIE's (8) nomenclature, the meristem will be called the meristem, and the tissues produced by it (the cicatrice and those between the cicatrice and the wound) the pseudocicatrice. In the pseudocicatrice there appears, later in the development of the cicatrice, some cells that lose their cell contents and are cut off from the living tissues but do not decompose and lose their identity. In the section (fig. 18) of the petiole that is attached to the plant there is a meristem formed before any of the decomposed tissue is apparent.

The meristem divides to form the cicatrice and cuts off a wall of living cells (fig. 20) back of the decomposed cells of the pseudocicatrice. The wall of living cells may be only one to seven cells wide. The pseudocicatrice has two types of cells, the decomposed cells of the first collapse and now the dead cells cut from the rest of the tissues by the cicatrice. The dead cells of the pseudocicatrice are easily distinguished and are without cell contents. The decomposed cells of the pseudocicatrice take a very dark stain characteristic of lignified tissue, while dead cells take a stain characteristic of suberized tissue. The outer cells of the cicatrice soon show the suberin reaction; in some cases the outer wall shows suberin as early as 2 days after they have been formed. The dead cells are thus cut off from water and food, and (fig. 20) become part of the pseudocicatrice.

After 4 days the meristem has up to four cell divisions. The leaf that re-

mained attached has more cells cut off in the pseudocicatrice (lightly shaded areas of figs. 21, 23, 25) and more divisions of the meristem.

After 6 days there is greater meristematic activity in the attached leaf than in the detached one (figs. 26, 28, 30, 27, 29, 31). Some of the cicatrice cells produced by the meristem have become suberized and no longer have cell contents. This is shown in the figures by the light shading. The cells of the bundle sheath of the petiole (fig. 31) have become meristematic and have grown in between the ends of the spiral elements of the xylem. In detached leaves the effect of drying out is evident. The cells have decreased in size and there is a swollen area where the meristem is active (figs. 27, 29). This swollen area was apparent in all detached leaves, no matter where the wound was made. It is not entirely due to the rigidity of the cicatrice and pseudocicatrice, for sometimes it is some distance behind the meristem, and a sunken area appears between the meristem and the cicatrice. There is apparently a water-storage tissue developed just back of the meristem, this tissue becoming more apparent as the drying continues (figs. 33, 35).

At the end of 6 weeks the cicatrice and many cells of the meristem have become suberized and are dead. There is some indication of lignin in the dead cells of the pseudocicatrice. The meristem has invaded the epidermis and produced a cicatrice sixteen cells away from the wound in some cases (figs. 32, 33, 34, 35). The sections of the detached leaf show the cells almost crushed (figs. 33, 35). In another week the whole leaf (except the petiole) is brittle and shatters at touch.

There is no evidence of any abscission of the pseudocicatrice or of the cicatrice. In fact, there is some evidence that the
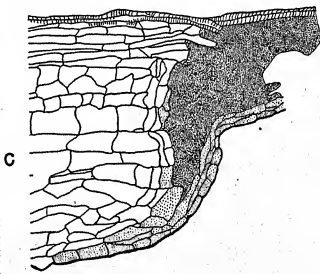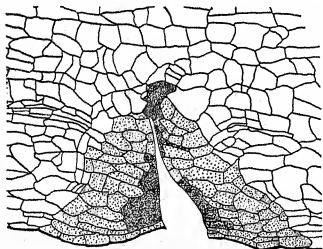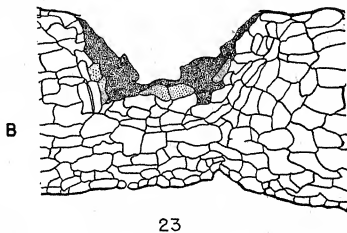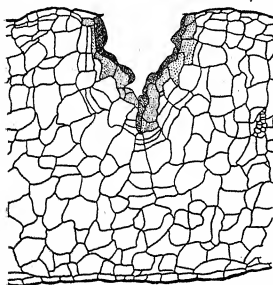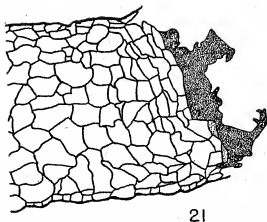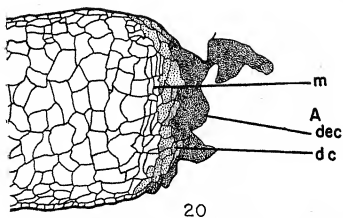
FIGS. 20–25.—Sections of leaves 4 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 20, attached leaf. Fig. 21, removed leaf. Fig. 22, attached leaf. Fig. 23, removed leaf. Fig. 24, attached leaf. Fig. 25, removed leaf. *dc*, dead cells of pseudocicatrice; *dec*, decomposed cells of pseudocicatrice; *m*, meristem.
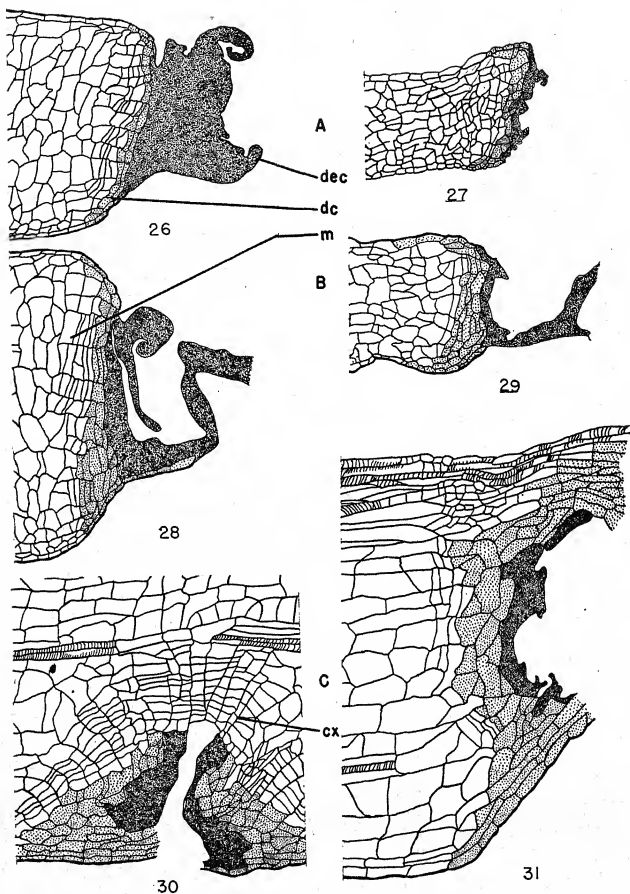
FIGS. 26–31.—Sections of leaves 6 days after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 26, attached leaf. Fig. 27, removed leaf. Fig. 28, attached leaf. Fig. 29, removed leaf. Fig. 30, attached leaf. Fig. 31, removed leaf. *cx*, cicatrice; *dc*, dead cells of pseudocicatrice; dec, decomposed cells of pseudocicatrice; *m*, meristem.
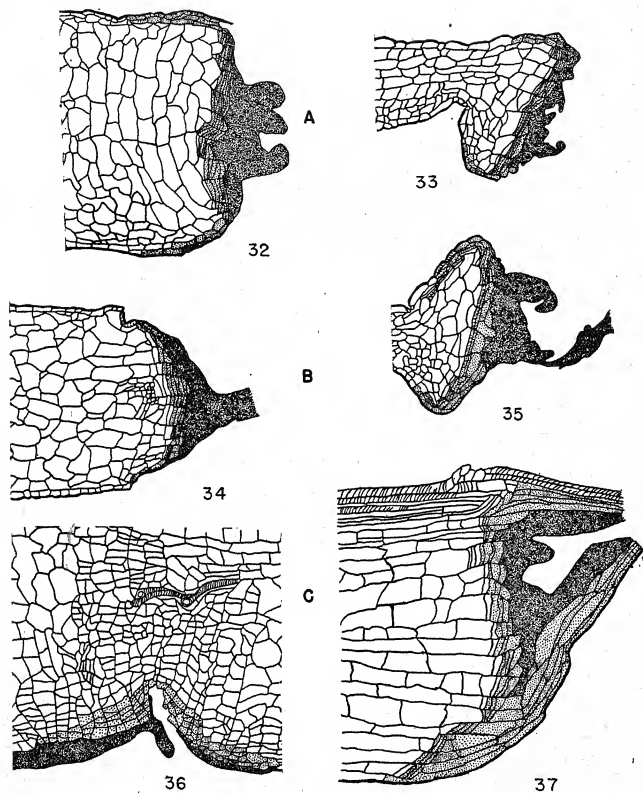
FIGS. 32–37.—Sections of leaves 10 weeks after wounds were made: *A*, marginal wounds; *B*, cross-hatch wounds; *C*, wounds of petioles. Fig. 32, attached leaf. Fig. 33, removed leaf. Fig. 34, attached leaf. Fig. 35, removed leaf. Fig. 36, attached leaf. Fig. 37, removed leaf.

meristem is so active that the wound will be completely filled with new tissue (fig. 36). This would seem to make the organ more solid than before activity started. Any dropping of tissue must have been due to the handling of the material and not from any natural abscission. More of the cicatrice tissue was developed in the leaf that was attached to the plant. If there is a water relation it might be shown here, since the leaf was allowed to dry at laboratory humidity.

The leaf that remained attached to the plant had more cells cut off between the meristem and the cells originally wounded. In this leaf there were twelve rows of cells between those of the original wound and the cell from which the meristem arose. In the detached leaf there were only eight cells at the most between the wound and the meristem. This would lead to the development of more pseudocicatrice in the leaf that remained on the plant.

Summary

1. The development of the cicatrice and the possibility of abscission of that tissue were studied in the leaves of *Bryophyllum calycinum*.

2. Stages in the development of the pseudocicatrice and the cicatrice were traced from wounds made in the leaf at the margin, in the center, and at the petiole of the leaf.

3. Two series of leaves were used. One series was attached to the plant and the other was removed and allowed to dry under laboratory conditions. There was greater collapse and greater development of the pseudocicatrice in the leaf that was attached than in the leaf removed from the plant.

4. There was no evidence of abscission, either from the leaves that were attached to or from those removed from the plant.

5. The idea that abscission of tissues developed in response to wounds if the moisture supply to the leaf is high does not apply to *Bryophyllum calycinum*.

6. Any loss of tissue must have come from mechanical agitation rather than from true abscission.

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MEGASPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF CYPRIPEDIUM PARVIFLORUM

MARGERY C. CARLSON

Literature review

A reinvestigation of megasporogenesis and the development of the embryo sac of *Cypripedium parviflorum* Salisb. seemed desirable because of the doubt expressed by some investigators concerning the so-called *Cypripedium*-type of embryo sac, which was based on PACE's (9) investigation of several species of this genus. SCHNARF (16) placed this type in his list of doubtful cases and called attention to the criticisms of PACE's work by RUTGERS (15). On the basis of RUTGERS' criticisms and of results of work on other species of *Cypripedium* by FRANCINI (5) and PROCINA (12), SCHNARF suggested that the embryo sac of *Cypripedium* develops according to the *Scilla*-type. MAHESHWARI (6, 7) agreed with SCHNARF and placed many members of the Orchidaceae in his *Allium*-type, which is the same as SCHNARF'S *Scilla*-type.

PACE (9) concluded from her study of *C. parviflorum, C. spectabile* (*C. hirsutum* Mill.), *C. pubescens* (*C. parviflorum* var. *pubescens* [Willd.] Knight), and *C. candidum* that the archesporial cell, which functions as the megaspore mother cell, divides by meiosis and forms two daughter cells. The micropylar cell disintegrates and the chalazal one continues its development, resulting in the formation of the embryo sac. The nucleus of the chalazal daughter cell divides into two nuclei (megaspore nuclei), but the cell does not divide. An enlarging vacuole in the center pushes the two nuclei to opposite ends of the cell, where a second division takes place, producing a 4-nucleate embryo sac. One micropylar nucleus becomes the egg, the other a synergid; one chalazal nucleus migrates to the micropylar end of the embryo sac and becomes the other synergid, while the second chalazal nucleus becomes the polar nucleus. One synergid is then pushed upward by the entering pollen tube and unites with the polar nucleus. After discharge from the pollen tube, one male nucleus unites with the egg and the other unites with the two polar nuclei in the center of the sac. This was one of the first instances in which the account of embryo-sac development deviated from what is usually regarded as the normal.

RUTGERS pointed out the following inconsistencies in PACE's work. PACE stated that the mature embryo sac before fertilization has four nuclei but figured one with five. She showed no intermediate steps in the passage of one synergid to the center of the sac, where, she said, it unites with the polar nucleus. RUTGERS stated that PACE simply inferred this interpretation and called attention to one of her figures in which the remains of two synergids can be seen after double fertilization has occurred. RUTGERS gave his interpretation of PACE's figures as follows: The daughters of the primary chalazal nucleus[1] could not have become synergid and egg because of the polarization of the embryo sac; the primary chalazal nucleus must divide late and its two daughter nuclei remain close together and later both unite with one of the male nuclei; the primary micropylar

[1] Terminology used in this paper is that suggested by MAHESHWARI.

nucleus divides, one daughter becoming the egg and the other dividing and forming the two synergids; one synergid disintegrates when the pollen tube enters. RUTGERS states, "We do not claim to have given a decision, this being impos-

TABLE 1

Species	Investigator and year
1. Ordinarily normal (monosporic and 8-nucleate):	
Orchis pallens..................................	Strasburger (19), 1878
†Gymnadenia conopsea...........................	Ward (22), 1880
Habenaria ciliaris (Michx.) R. Br................	Brown (2), 1909
H. integra (Nutt.) Spreng......................	" "
Calopogon pulchellus R. Br.....................	Pace (10), 1909
†*Epipactis pubescens (Willd.) A. A. Eaton.........	Brown and Sharp (3), 1911
*Epipactis latifolia............................	Vermoesen (21), 1911
*Epidendrum variegatum Hook....................	Sharp (17), 1912
E. verrucosum Sw..............................	" "
E. cochleatum L...............................	" "
E. globosum Jacq..............................	" "
Bletia shepherdii Hook.........................	" "
Coelogyne massangeana.........................	" "
Pogonia macrophylla...........................	" "
*Gyrostachis cernua............................	Pace (11), 1914
G. gracilis...................................	" "
*Orchis morio L................................	Afzelius (1), 1916
*Orchis sambucina L............................	" "
Oncidium praetextum Rchb. fil..................	" "
Coeloglossum viride (L.) Hn....................	" "
Gymnadenia albida (L.) L. C. Rich..............	" "
Goodyera repens (L.) R. Br....................	" "
Neottia nidus-avis.............................	Modilewski (8), 1918
Moringa oleifera Lam..........................	Puri (13), 1935
Garcinia livingstonii T. Anders.................	Puri (14), 1939
2. Monosporic, normally producing less than 8-nuclei in embryo sac:‡	
Garcinia kydia Roxb...........................	Treub (20), 1911 (doubted by Puri [14], 1939)
G. treubii Pierre..............................	" "
Phajus grandifolius Lour.......................	Sharp (17), 1912
Corallorhiza maculata Raf......................	" "
Broughtonia sanguinea R. Br...................	" "
3. Normally bisporic, thus belonging to Maheshwari's Allium-type:	
Cypripedium parviflorum.......................	Pace (9), 1907
C. spectabile (C. hirsutum Mill.)..............	" "
C. pubescens (C. parviflorum var. pubescens [Willd.] Knight)...................................	" "
C. candidum..................................	" "
†Paphiopedilum insigne (Wall.) Pfitz.............	Afzelius (1), 1916
†Cypripedium guttatum Sw......................	Procina (12), 1930
Paphiopedilum leeanum........................	Francini (5), 1931₆
†P. spicereanum (Rchb. fil.) Pfitz..............	" "
†P. barbatum (Lindl.), Pfitz...................	" "
†P. villosum (Lindl.), Pfitz...................	" "
†P. venustum (Wall.), Pfitz...................	" "
Achroanthes monophyllos (L.) Greene...........	Stenar (18), 1937

* A few of these embryo sacs reported bisporic; a few tetrasporic.

† Some mature embryo sacs may have less than 8 nuclei, either because the antipodal nuclei degenerate very early or because daughter nuclei of the primary chalazal nucleus do not divide.

‡ In these the chalazal nuclei do not divide after the 4-nucleate stage of the embryo sac, which is therefore 6-nucleate when mature.

sible without a reinvestigation of the whole material. But our suggestions must be admitted as a possible explanation and must be rejected on firm grounds before we can accept Miss PACE's."

RUTGERS described the development of the embryo sac in the orchid *Moringa oleifera* Lam. He found four megaspores in a T-arrangement, only one of which functions in forming the embryo sac. Two nuclear divisions occur normally, but the third division is restricted to one of the micropylar nuclei. The mature sac has, therefore, two chalazal nuclei which unite and become the secondary (fusion) nucleus, and three micropylar nuclei, two of which are synergids and one the egg. This sequence of events is similar to RUTGERS' suggestion of possible events in *Cypripedium*. However, PURI (13), reinvestigating *M. oleifera*, found the normal eight nuclei in the mature sac.

TREUB (20) described the course of development of the embryo sacs of *Garcinia kydia* Roxb. and *G. treubii* Pierre, and RUTGERS, probably influenced by TREUB, found the same course in *Moringa;* but PURI (14) found the normal eight nuclei in the mature sac of *G. livingstonii* T. Anders. Table 1 gives a classification of the work on the embryo sac of orchids.

Material and method

The material used in this work was collected and prepared primarily for a study of seed formation, as described previously by CARLSON (4). Young flower buds were bagged, and when they had opened and their stigmas were receptive, they were pollinated and then rebagged. Ovaries were collected at intervals and were prepared for sectioning by the usual paraffin technique and were stained with safranin and fast green.

The relation of the stage of develop-

ment of the ovules and their contents to the season and to the date of pollination was given in the earlier paper and will not be repeated here.

Observations

CHROMOSOMES

PACE (9) reported eleven pairs of chromosomes at the equator of the spindle in the metaphase of meiosis. I find ten pairs, as seen in figures 3 and 4. Many counts were made, and in no case where the chromosomes were sufficiently spread out to make the count certain were more than ten pairs found. The chromosomes are short and thick and vary somewhat in size and form. Even though they are small, it was possible to distinguish chromonemata and matrix.

MEGASPOROGENESIS

The single hypodermal archesporial cell enlarges greatly and its nucleus undergoes the first meiotic division, thereby functioning directly as the megaspore mother cell (fig. 1). A prophase, metaphase, and telophase of this division are seen in figures 1, 2, and 5, respectively. When the two daughter nuclei are reconstructed, a wall is formed dividing the mother cell into two approximately equal-sized daughter cells—the dyads (fig. 6). The chalazal dyad begins to enlarge and the micropylar one soon shows signs of disintegration (fig. 7). The nucleus of the chalazal dyad now undergoes the second division, but the cell does not divide (figs. 7, 8). Many stages of this nuclear division were found, but no indication of even an ephemeral wall was seen. The two nuclei in the dyad, the result of two divisions following the megaspore mother nucleus, are equivalent to megaspore nuclei. Both take part in the formation of the embryo sac, making it bisporic.
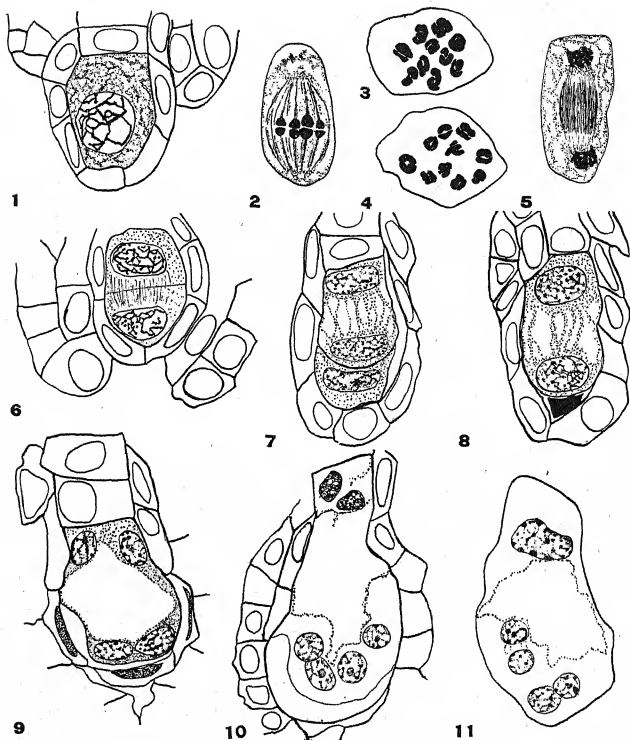
Figs. 1–11.—Camera lucida drawings. All figures arranged so that chalazal end of ovule or embryo sac is toward top of page. Fig. 1, tip of ovule with lobe of inner integument showing; nucleus of enlarged megaspore mother cell in prophase of meiosis. Fig. 2, lateral view of heterotypic metaphase; only four of the ten pairs of chromosomes seen. Figs. 3, 4, polar views of heterotypic metaphase, showing ten pairs of chromosomes. Fig. 5, telophase of heterotypic division. Fig. 6, nuclear division completed; cell plate forming; each cell will be a dyad; lobe of inner integument extending around nucellus. Fig. 7, chalazal dyad elongated and its nucleus divided into two megaspore nuclei; micropylar dyad somewhat crushed. Fig. 8, chalazal dyad more elongated but no indication of cell division; vacuole developing between the two nuclei and initiating formation of bisporic embryo sac; micropylar dyad disintegrated. Fig. 9, each nucleus of embryo sac divided; micropylar dyad completely disappeared. Fig. 10, each nucleus at micropylar end of sac divided; those at chalazal end not divided and staining more heavily with safranin. Fig. 11, four nuclei at micropylar end of embryo sac; two nuclei at chalazal end have united.

EMBRYO SAC

A vacuole appears between the two nuclei in the chalazal dyad, which now becomes the embryo sac. As the vacuole enlarges, the embryo sac elongates and its nuclei are pushed to the ends of the sac (figs. 7, 8). Both nuclei divide, making four (fig. 9). Up to this point my ob-

servations agree with those of PACE, but beyond the 4-nucleate stage the events differ from those described by her.

The two nuclei at the micropylar end of the embryo sac divide and the four daughters lie free in the cytoplasm. The two chalazal nuclei, however, do not divide (fig. 10). This 6-nucleate stage was
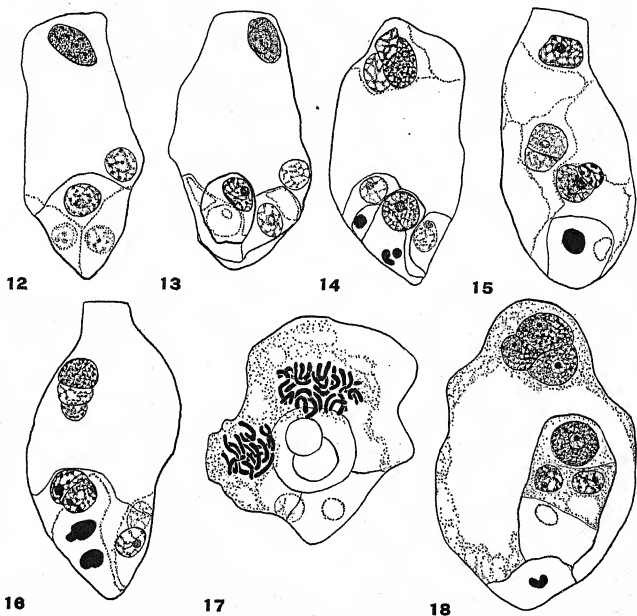
FIGS. 12–18.—Figs. 12, 13, egg and two synergids formed; polar nucleus free in inner cell; chalazal nuclei united. Fig. 14, end of pollen tube, with tube nucleus and two sperms, in embryo sac; two chalazal nuclei and polar nucleus lying in contact. Fig. 15, double fertilization; one sperm in contact with egg, other with polar nucleus; fused chalazal nuclei at inner end of sac. Fig. 16, double fertilization; one sperm in contact with egg; second sperm, polar nucleus, and fused chalazal nuclei in contact (latter group [4n] functions as primary endosperm nucleus). Fig. 17, polar view of embryo sac; 3-celled embryo; division of two groups of primary endosperm nuclei. Fig. 18, young embryo with 3-celled embryo proper and 1-celled suspensor; fused chalazal nuclei, polar nucleus and sperm in contact; pollen tube with tube nucleus still visible but synergids have disappeared.

missed by PACE, but it appears frequently in the present material.

Three of the micropylar nuclei now form the typical egg apparatus, and the fourth (upper polar) moves to the center of the sac (figs. 12, 13). The two chalazal nuclei may remain apart, they may approach and lie in contact, or they may fuse (figs. 12, 13). Sometimes they unite before the egg apparatus is constructed (fig. 11), and sometimes they are still apart after the embryo has started to form. The upper polar nucleus may fuse with the two chalazal nuclei but usually simply lies in contact with them (fig. 14).

FERTILIZATION

The pollen tubes grow downward on either side of the base of each of the three branching placentae. Great masses of them follow along the surface of the placentae. They reach the ovules when meiosis is in progress or during the early stages of embryo-sac development. The tube nucleus and the two male nuclei follow in succession down the tube and may easily be found just back of its tip. When a pollen tube enters the embryo sac (fig. 14) it sometimes becomes difficult to find the synergids (fig. 15), but often both may be found as late as fertilization (fig. 16). The tube and male nuclei change their shapes and staining reactions when the tube enters the sac (fig. 14). After discharge from the tube, the male nuclei enlarge and migrate into the sac, where one unites with the egg (figs. 15, 16).

The history of the second sperm depends on what has happened in the lower end of the sac. As already described, the two chalazal and the upper polar nuclei may not actually fuse but merely lie in contact, or the two chalazal nuclei may fuse and this double nucleus may

lie in contact with the upper polar one. In either case the second sperm usually moves into contact with, but does not fuse with, the group (figs. 16, 18). Sometimes the polar nucleus does not join the chalazal nuclei but unites with the sperm independently (figs. 15, 17). There is no triple-fusion nucleus in the ordinary sense of the term. Because this group of nuclei is concerned with the formation of endosperm, it may be called the primary endosperm nucleus or group of nuclei. Frequently, one or two dark-staining bodies remain in the pollen tube after fertilization (fig. 16).

EMBRYO AND ENDOSPERM

The early stages of embryo development have already been described by CARLSON and by PACE, but 3- and 4-celled stages may be seen in figures 17 and 18. The primary endosperm nucleus, or group of nuclei, contains a tetraploid chromosome complement. PACE shows a metaphase and telophase in the first division of the primary endosperm nucleus and also illustrates embryo sacs with two and four large endosperm nuclei. In most of the sacs in my material the nuclei, which should form the primary endosperm nucleus, neither fuse nor divide, but figure 17 shows an end view of an embryo sac with metaphases or early anaphases of two nuclei which started to divide before uniting. The embryo in this figure is in the 3-celled stage. Whether or not the primary endosperm nucleus divides, it or its descendants soon disintegrate and the mature seed has no endosperm.

Discussion

This study confirms PACE's conclusion that two megaspore nuclei enter into the formation of the embryo sac, but corrects

her ideas about the fate of the nuclei after the 4-nucleate stage of the sac. The micropylar nucleus of the dyad functions normally, but the one at the opposite end divides although its daughters do not. They are variable in their behavior, usually fusing and together functioning as the upper polar nucleus. There are no antipodal cells as ordinarily defined. Fertilization of the egg is normal, but the other "fertilization" is variable. The primary endosperm nucleus, or group of nuclei, which is 4n, may or may not divide, forming endosperm nuclei. RUTGERS' guess that the daughters of the primary chalazal nucleus fuse and then unite with a male nucleus was right, but he was mistaken in thinking that no polar nucleus was formed in the micropyler end of the sac. Variability in the chalazal end of the sac is not unusual. It occurs in those plants marked with † in table 1.

Most of the orchids which have been investigated have normal, monosporic, 8-nucleate embryo sacs, but some are monosporic with 6-nucleate sacs. *Cypripedium parviflorum*, *C. hirsutum*, *C. pubescens*, *C. candidum* (9), *C. guttatum* (12), *Achroanthus monophyllas* (18), and six species of *Paphiopedilum* (1, 5), are bisporic and 6-nucleate. The so-called *Cypripedium*-type of embryo-sac development must be abandoned and the bisporic types placed in the *Scilla*-type of SCHNARF, or the *Allium*-type of MAHESHWARI, who claims that the name "*Allium*-type" has priority.

Summary

1. The haploid number of chromosomes in *Cypripedium parviflorum* is ten rather than eleven, as previously reported.

2. Two potential megaspore nuclei enter into the formation of the embryo sac, making it bisporic, and placing this species of *Cypripedium* in the *Scilla*-type of SCHNARF, or the *Allium*-type of MAHESHWARI.

3. After the 4-nucleate stage of the embryo sac, the two micropylar nuclei divide and two of the resulting four nuclei become synergids, one becoming the egg and the other the polar nucleus. The two chalazal nuclei do not divide and usually fuse. They may, however, simply lie in contact. At this stage the embryo sac contains either six or five nuclei, depending on whether the chalazal nuclei have fused.

4. The polar nucleus from the micropylar group usually migrates into contact with the fused or contiguous chalazal nuclei and may never actually fuse with them.

5. Double fertilization occurs. One sperm fuses with the egg and the second may unite with or lie in close contact with the chalazal group of nuclei.

6. The primary endosperm group of nuclei, containing the tetraploid number of chromosomes, may not divide or it may reach a few-nucleate stage before disintegrating.

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EFFECT OF COMMERCIAL FERTILIZERS ON THE SEX EXPRESSION OF HEMP[1]

C. A. BLACK

Introduction

Owing to the shortage of hard fibers in the United States during the early part of the war, hemp was grown in several states for military and domestic needs. Except for Kentucky and Wisconsin, hemp had not been grown previously to any extent in the states selected for production, and relatively little was known about the successful culture of the crop in the new areas. For this reason, a number of field experiments with commercial fertilizers were undertaken to determine their value in hemp production. These experiments, while designed primarily to obtain data on yield,

furnished a good opportunity to study the effect of fertilizers on the sex ratio of hemp under field conditions. Practical interest in the problem arises from the difference in the nature and quantity of fiber obtained from plants of the different sexes. The male plants contain a harsher fiber and a higher percentage of it than do the female plants (3). The present paper records the results of sex counts obtained from experiments conducted in Iowa in 1943 and 1944.

Review of literature

Several investigators have found it possible to bring about a change in the sex expression of certain plants by varying the environmental conditions. In the case of hemp, the factors of light, tem-

perature, and nutrient supply have been shown to be of importance. By varying the photoperiod, SCHAFFNER (8) was able to control the degree of sex reversal. Where the length of the photoperiod was short, more than 90% of the plants showed sex reversal, the male to the female and vice versa. NELSON (6) noted that in both wild and fiber hemp, low temperature ($15°$ C.) favored a dominance of female plants. The effect was more pronounced when the aerial portion of the plant was subjected to the low temperature than when the roots were kept at the low temperature.

No particular effect of nutrition was noted by earlier investigators. The data reviewed by HEYER (5) were negative. It was concluded, therefore, that sex was determined in the seed and not influenced by external conditions.

BOSE (2), working with hemp, reported some counts made in a portion of a field which had been fertilized with mustard cake (an organic fertilizer containing nitrogen, together with some phosphorus and potassium). The relative numbers of males and females were almost identical on the treated and untreated areas. Additional counts were made on a field of four plots, three receiving commercial fertilizers and one serving as a check. The ratio of males to females varied from 1:0.92 on the check plot to 1:1.27 on the ammonium-sulphate plot. Superphosphate and Ammophos gave intermediate values. Although the differences probably are within the limits of sampling variation, the hemp on all the fertilized areas had a smaller proportion of males than had the check; furthermore, both the plots receiving nitrogen had a lower percentage of males than had the two plots not fertilized with nitrogen.

Later work by TIBEAU (10), conducted in the greenhouse, showed that by varying the nitrogen supply it was possible to produce all male, all female, or a population of male and female plants. When no nitrogen was supplied, all the plants were male. When the plants were grown with a "normal" supply (that furnished in Knop's solution), 65% of the plants were female and 35% were male. With eight times the "normal" concentration of nitrogen, all the plants were female.

The results of TIBEAU (10), in conjunction with those of TALLEY (9), GARDNER (4), and others, suggest that the carbohydrate-nitrogen balance in certain plants influences the sex expression. It seems that, in general, high-nitrogen low-carbohydrate plants tend toward femaleness, whereas low-nitrogen high-carbohydrate plants tend toward maleness.

Experimental methods

In 1943, the relative numbers of male and female plants were counted in two experiments. The treatments employed were commercial ammonium sulphate, superphosphate, and potassium chloride added at rates to furnish, respectively, N at 25 pounds per acre, P_2O_5 at 50 pounds per acre, and K_2O at 25 pounds per acre. Each treatment was used singly and in all combinations with each of the others in a factorial design having four replications. In 1944, counts were made on six experiments. The 1944 experiments included N at 50 and 100 pounds per acre, P_2O_5 at 30 pounds per acre, and K_2O at 20 pounds per acre, again in a factorial design with four replications (three replications in experiments 12 and 13).

The fertilizers were applied on small plots, 10×13 feet in 1943 and 13×16 feet in 1944. The sample of hemp taken from each plot consisted of a composite

of six 24 × 26 inches of sub-samples in 1943 and of five 36 × 36 inches of sub-samples in 1944. In making the sex counts, small lots of about ten plants each were taken at random from the composite sample. The male and female plants were counted before another lot was taken. This process was repeated until a total of 100 male plus female plants had been counted per plot. No

the percentage of female plants represents the difference between that and 100.

Results and discussion

In both 1943 and 1944 it was found that fertilizers affected the yield of hemp. In general, nitrogen was the major limiting factor, followed in order by phosphorus and potassium. Table 1 summarizes the effects of fertilizers on the yield

TABLE 1

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM
FERTILIZERS ON YIELD OF GREEN HEMP

FIELD NO.*	YIELD WITH-OUT FER-TILIZER (TONS PER ACRE)	AVERAGE YIELD INCREASE WITH TREATMENT† (TONS PER ACRE)			
		N		P	K
3.............	8.21	1.99‡		0.79	−0.94
6.............	7.02	2.17‡		0.37	−0.59§
		N_1	N_2	P	K
10.............	10.16	2.81‡	4.06‡	−0.26	0.54§
11.............	10.54	2.90‡	3.57‡	−0.46	0.64
12.............	8.73	2.21‡	2.98‡	0.58§	0.26
13.............	11.23	1.11‡	1.63‡	0.37	0.20
14.............	14.32	0.91	1.38‡	1.07‡	−0.47
15.............	3.25	0.52	1.23‡	−0.09	−0.24

* For further details regarding yields, soil types, past management, location, etc., see BLACK and VESSEL (1).

† Acre applications of fertilizer ingredients as follows: For fields 3 and 6: N 25 lb. N; P 50 lb. P_2O_5; K 25 lb. K_2O. For fields 10–15: N_1 50 lb. N; N_2 100 lb. N; P 30 lb. P_2O_5; K 20 lb. K_2O.

‡ Significant at 1% level.

§ Significant at 5% level.

hermaphroditic specimens were noted, although there may have been a few which escaped attention. In each experiment it was found necessary to discard a few plants which were not far enough advanced to be recognized definitely as either male or female. These were small plants which presumably germinated late and were stunted by lack of light under the foliage of the earlier plants.

The results of the counts are expressed in this report in terms of the percentage of male plants, it being understood that

of hemp for those experiments on which counts of male and female plants were made.

. The results of counts made on the 1943 experiments are shown in table 2. It will be noted that about half the plants were male, regardless of treatment. The only difference which reached significance at the 5% level was that for phosphorus on field 6, where hemp on phosphorus-treated plots averaged 52% male and that without phosphorus averaged 48%. In the final yields on field 6 there was no

significant response to phosphorus. Early in the season, however, definite response to phosphorus was evident. The reverse trend in percentage of male plants was found on field 3, where there was more marked early response to phosphorus and where phosphorus produced twice as much increase in the final yields.

The hemp on both field 3 and field 6 was rather low-yielding, owing in the main to a deficiency of nitrogen. Even the nitrogen-fertilized hemp was defi-

a value well within the range obtained on the adjacent experiment.

One replicated experiment was conducted in 1943 in which nitrogen was applied at the rate of 100 pounds per acre to a field which gave a high yield of hemp without fertilization[3] and on which the nitrogen-fertilized hemp gave a test for nitrate nitrogen throughout the season. Counts made on composite samples from all replications gave 50% male on the hemp without additional nitrogen and

TABLE 2

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZERS
ON PERCENTAGE OF MALE HEMP PLANTS; 1943 EXPERIMENTS

FIELD NO.	PERCENTAGE MALES WITH TREATMENTS*								Average effects					
	None 0-0-0	N 5-0-0	P 0-10-0	NP 5-10-0	K 0-0-5	NK 5-0-5	PK 0-10-5	NPK 5-10-5	N	N_1	P_0	P_1	K_0	K_1
3..........	56.0	52.0	52.0	47.0	49.0	48.0	47.0	48.0	51.0	48.7	51.2	48.5	51.7	48.0
6..........	47.7	49.2	50.0	52.7	46.0	49.0	52.2	53.0	49.0	51.0	48.0	52.0†	49.9	50.1
Average....	51.9	50.6	51.0	49.9	47.5	48.5	49.6	50.5	50.0	49.9	49.6	50.2	50.8	49.0

* 500 pounds of each fertilizer per acre.
† Significant difference at 5% level.

cient throughout a considerable part of the growing season. To obtain some indication as to the effect of a greater supply of nitrogen, counts were made on a heavily fertilized, non-replicated strip at the side of experiment 3. The fertilizers applied to this area included phosphorus and potassium at the same rates as those used in the experiment, and nitrogen at the rate of 100 pounds per acre—four times the rate in the experiment. Hemp on this more heavily fertilized area did not contain discernible quantities of nitrate nitrogen after midseason, but should have had an adequate supply earlier in the season.[2] Sex counts showed the presence of 49% male plants,

[2] Acre yield of green hemp was 14.87 tons.

49% on the hemp which received the heavy application of nitrogen.

The results of counts made on the 1944 experiments are shown in table 3. Since the 25-pound rate of nitrogen used in 1943 was clearly inadequate in many cases, nitrogen was applied at 50 and 100 pounds per acre in 1944, with the assumption that 100 pounds would be adequate on the poorer fields and excessive on the better fields. As a result of heavy rains in the spring and early summer, fields 12, 13, and 15 were not planted for some time after the fertilizers had been

[3] Acre yield of green hemp was 16.85 tons on the plots without nitrogen and 21.20 tons on the plots which received nitrogen at the rate of 100 pounds per acre.

TABLE 3

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZERS ON PERCENTAGE OF MALE HEMP PLANTS; 1944 EXPERIMENTS

PERCENTAGE MALES WITH TREATMENT (500 POUNDS FERTILIZER PER ACRE)

FIELD NO.	None 0-0-0	N_1 10-0-0	N_2 20-0-0	P 0-6-0	N_1P 10-6-0	N_2P 20-6-0	K 0-0-4	N_1K 10-0-4	N_2K 20-0-4	PK 0-6-4	N_1PK 10-6-4	N_2PK 20-6-4	N_0	N_1	N_2	P_0	P_1	K_0	K_1
10	50.0	49.0	53.5	50.2	47.5	42.5	49.0	48.2	50.5	49.5	50.2	44.7	49.7	48.7	47.8	50.0	47.5†	48.8	48.7
11	55.0	46.0	57.7	55.5	55.0	50.7	58.7	49.2	48.7	59.0	48.5	48.5	54.8	49.7	51.4	52.6	51.4	53.3	50.6
12	48.0	52.0	49.3	50.0	49.0	43.0	51.0	44.0	43.7	46.7	47.7	50.3	48.9	48.2	46.6	48.0	47.8	48.6	47.2
13	51.0	47.7	50.3	50.0	46.3	50.3	51.0	49.0	49.0	51.3	49.3	42.7	50.9	48.1	49.7	49.7	48.4	49.3	48.7
14	45.0	44.2	46.2	41.2	44.0	44.7	43.5	48.7	48.0	51.0	48.5	50.7	45.2	46.4	47.4	46.0	46.7	44.2	48.4*
15	46.7	44.7	45.0	44.0	49.5	47.2	46.2	51.7	45.0	41.2	48.2	47.0	44.6	48.1	46.1	46.6	45.9	46.2	46.2
Average‡	50.2	46.7	52.1	49.3	48.3	46.9	50.5	48.8	49.1	50.4	49.1	46.9	50.1	48.2	48.7	49.6	48.5	48.9	49.1
Average§	49.3	47.0	50.4	48.4	48.6	46.4	49.8	48.7	47.6	48.2	48.4	47.4	48.9	48.2	48.0	48.8	47.9	48.4	48.4

(under the group "Average effects": columns N_0, N_1, N_2, P_0, P_1, K_0, K_1)

* Difference significant at 1% level.

† Difference significant at 5% level.

‡ Omits fields 12 and 15, on which the nitrogen-fertilized hemp was deficient in nitrogen. § Includes all fields. Experiments 12 and 13 each had three replications, whereas all others had four.

Each replication has been given equal weight in the averages, which gives experiments 12 and 13 somewhat less weight than the others.

applied. Apparently there was opportunity for loss of some of the nitrogen by leaching before the hemp was planted, because even the hemp on plots receiving 100 pounds of nitrogen per acre was deficient in nitrogen on fields 12 and 15. Because of this difference in the nitrogen status of the different experiments, the data have been averaged in two groups, one including only those experiments (10, 11, 13, and 14) where the nitrogen-fertilized hemp had an adequate supply of nitrogen and the other including all experiments.

Again in 1944 all treatments averaged about 50% male (the average of all experiments was 48.4%). There was a slight tendency for the percentage of males to decrease as both nitrogen and phosphorus were increased. The lowest percentages occurred on the plots which received both nitrogen (at the 100-pound rate) and phosphorus. The analysis of the combined data from all six experiments indicated a significant interaction of nitrogen and phosphorus.

Statistically significant differences occurred in two individual experiments. On field 10, the phosphorus-treated plots had significantly fewer males. The depression in number of males occurred entirely at the highest level of nitrogen (N_2) and resulted in a highly significant N-P interaction. On field 14 there was a highly significant increase in the percentage of male plants on the plots which received potassium. The increase from potassium was four times as great in the presence of phosphorus as in the absence of phosphorus, a significant interaction.

Field 10 gave an increase in acre yield of 4.06 tons from nitrogen and 0.54 tons from potassium, and a decrease of 0.26 tons from phosphorus. The acre yield of hemp on field 14 was increased 1.38 tons from nitrogen and 1.07 tons from phos-

phorus, and was decreased 0.47 tons from potassium. Thus, nitrogen, which had the greatest effect on the yield of hemp and might be expected to have the greatest effect on the relative numbers of male and female plants, was the only fertilizer constituent which produced no significant main effects. The fertilizers which significantly affected the relative numbers of male and female plants (phosphorus in experiment 10 and potassium in experiment 14) were ingredients which had little or no effect on the yield. The same may be said for the 1943 results.

No physiological explanation for the results obtained is apparent, and yet it would be unusual to obtain three statistically significant main effects in eight experiments purely as a matter of chance. The changes were small, however, and the effects were of no practical importance· in producing hemp consisting largely of either male or female plants. The conditions necessary to produce hemp of this nature evidently lie outside the range produced in these experiments.

As regards the use of nitrogen for producing a stand of female plants, it would appear that the quantity required under field conditions would be extremely high. Moreover, present indications are that weak fiber would be obtained from such high-nitrogen plants. On the other hand, to produce a preponderance of male plants by withholding nitrogen apparently would require such marked nitrogen deficiency that only a very low yield of fiber could be obtained.

Summary

Eight field experiments with hemp were conducted in Iowa in which was determined the effect of nitrogen, phosphorus, and potassium fertilizers on the relative numbers of male and female plants. Phosphorus produced a statisti-

cally significant increase in percentage of male plants in one experiment and a decrease in another. Potassium produced a significant increase in percentage of male plants in one experiment. No significant effects were produced by nitro-

gen. All differences due to fertilizers were small, the sex ratios varying but little between treatments.

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EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE READILY AVAILABLE CARBOHYDRATE CONSTITU- ENTS IN ANNUAL MORNING-GLORY

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Introduction

The use of certain growth-regulating substances as herbicides has recently been considered (1, 2, 3, 4, 7, 8, 9). It has been reported that the physiological changes associated with the death of plants treated with toxic amounts of 2,4-dichlorophenoxyacetic acid and some other related compounds occur at a relatively slow rate, so that the plants may

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live for 2–4 weeks after treatment (3, 4, 7).

In earlier experiments, naphthaleneacetic acid and some other compounds accelerated the rate of starch hydrolysis and depleted the supply of readily available carbohydrate reserves in bean leaves when these substances were applied as emulsion sprays (5, 6).

The present experiments were undertaken to determine the effect of spray application of toxic amounts of 2,4-dichlorophenoxyacetic acid on the readily available carbohydrate reserves of an-

nual morning-glory plants, *Ipomoea lacunosa* L., some of which were grown under conditions of photoperiod and soil fertility favoring vigorous vegetative growth and others under less favorable conditions.

Experimentation

PRELIMINARY TEST

In a preliminary experiment, morning-glory plants were grown in composted soil contained in 4-inch clay pots under greenhouse conditions, during the months of January, February, and March, 1945. During this period the plants grew only a limited amount vegetatively but produced numerous flowers and seeds. Approximately 2 months after planting, the plants were 4–6 inches tall, bushy, and had matured four to ten seedpods per plant. One hundred uniform plants were selected, and two-thirds of them were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500. The foliage of each plant was thoroughly covered with this solution. The remaining plants were left untreated as controls. Samples consisting of three treated and three untreated plants of average size were collected at intervals of 2–4 days.

Microchemical estimations of the amount of starch in the plants were made by cutting transverse sections of stem tissue at one-fourth and at one-half the distance from the apex to the ground level, at the ground level, and also a section was cut through the upper part of the root. These sections were placed in iodine solution and the amount of coloration due to the starch content in the tissue was noted.

Two weeks after treatment, microchemical tests indicated that the starch reserve had greatly decreased in the stems of treated plants. At the end of 3 weeks only a trace remained in these stems, while those of untreated plants contained an abundance of starch. At this time the leaves of the treated plants were brown and curled, the stems were beginning to dry, and the plants were near death. The untreated plants were green and healthy in appearance, although they were not growing vegetatively.

NONVEGETATIVE PLANTS

METHODS.—Morning-glory plants were grown from seed under greenhouse conditions in lightly composted soil contained in 4-inch clay pots. Small pots and soil of low fertility were used, in order to provide conditions unfavorable for vigorous vegetative growth. On March 20 the plants were 3–6 inches tall, and 330 were selected for size and uniformity. One-half of these were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. The foliage was thoroughly covered with the spray mixture. In order to inhibit excessive vegetative growth and hasten fruit production, both treated and untreated plants were covered with a black cloth so as to permit but 10 hours of natural illumination.

Thirty treated and thirty untreated plants were harvested at intervals during the next 20 days. Plants in these samples were divided into roots, hypocotyls, stems, flower buds, and leaves, and all like parts were combined to make five individual samples for each treatment. The samples were dried at 80° F., ground, and analyzed for total reducing sugars and for dextrin and starch by means of saliva digestion (6).

RESULTS.—Within 24–48 hours slight epinasty and stem curvatures developed on treated plants. Treatment inhibited vegetative growth (table 1), and the leaves of the sprayed plants became yellow within 2 weeks after treatment. New flower buds failed to develop on the treated plants, and those that were partially developed at the time of treatment became discolored and died within 6–10

TABLE 1

FRESH WEIGHT OF UNTREATED MORNING-GLORY PLANTS COMPARED WITH THAT OF PLANTS SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES EXPRESSED IN GRAMS AND OBTAINED FROM SAMPLES OF THIRTY TREATED AND THIRTY UNTREATED GREENHOUSE PLANTS

PLANT PARTS		DAYS AFTER TREATMENT			
	0	8		20	
		Con-trol	Treat-ed	Con-trol	Treat-ed
Roots.......	31.5	24.6	33.3	38.0	12.0
Hypocotyls...	8.0	8.6	9.0	8.3	5.6
Stems......	24.1	23.6	25.4	29.0	14.4
Buds........	6.7	16.1	7.2	35.0	1.7
Leaves.......	71.0	57.0	60.0	40.0	16.1
Total......	141.3	129.9	134.9	150.3	49.8

days. The flower buds were the first part to show necrosis as a result of treatment and the first to die.

Thirty untreated plants developed 130 mature fruits and forty-four immature ones during the experiment, while treated plants failed to develop fruit. Twenty days after treatment, the foliage and stems of the sprayed plants were discolored, on many of the plants a region of stem near the soil surface was partially decomposed as a result of the growth of fungi and bacteria in the stem tissues, and the plants were designated as dead

(fig. 1). Ninety-five per cent of the roots of the sprayed plants were classified as dead 20 days after treatment, and the remaining ones appeared beyond recovery.

Untreated plants failed to increase appreciably in size during the experiment, since they were subjected to environmental conditions which did not favor vigorous growth (table 1). The average dry weight of the treated plants decreased by approximately 60%, which may have been due in part to digestion by microörganisms during the latter part of the experiment.

The sugar content of sprayed plants increased markedly up to about the eighth day, when they contained 73% more sugar than did the controls; then the sugar content of the sprayed plants decreased, until at the end of the experiment they contained only about one-third as much sugar as did the unsprayed ones (table 2). The most marked increase in sugar content as the result of treatment occurred in the leaves. In contrast, the sugar content of the flower buds of the treated plants remained consistently below that of buds of the untreated ones. In treated plants no sugar could be detected in the tissues of the buds, and there was only a trace of it in the roots on the twentieth day following treatment.

The starch-dextrin content of the sprayed plants decreased markedly, and on the twentieth day following treatment they contained only about one-fifteenth as much starch and dextrin as did the unsprayed plants. The most rapid rate of hydrolysis of starch occurred in the leaves, buds, and stems in the treated plants (table 2).

The percentage of readily available carbohydrates (sugar, starch, and dextrin) decreased in the untreated and

Fig. 1.—Morning-glory plants subjected to 10-hour photoperiod and grown in relatively infertile soil to inhibit vegetative growth. Untreated (left) compared with plants sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (right). Twenty-one days after treatment.

TABLE 2

TOTAL SUGAR AND STARCH-DEXTRIN CONTENTS OF MORNING-GLORY PLANTS GROWN IN GREEN-HOUSE UNDER CONDITIONS OF PHOTOPERIOD AND SOIL FERTILITY UNFAVORABLE FOR VIGOROUS VEGETATIVE GROWTH AND SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES REPRESENT MILLIGRAMS OF CARBOHYDRATES IN THIRTY PLANTS

Days	Roots C*	Roots T	Hypocotyls C	Hypocotyls T	Stems C	Stems T	Buds C	Buds T	Leaves C	Leaves T	Total C	Total T
							Sugar					
0	288	.....	36	.....	139	.....	36	.....	190	.....	689	.....
3	216	228	49	87	144	230	46	33	260	660	715	1238
8	232	200	56	183	138	322	197	51	133	281	756	1037
15	136	38	55	152	118	191	262	10	46	43	617	434
20	170	6	30	98	86	100	349	0	128	17	763	221
							Starch-dextrin					
0	778	.....	713	.....	925	.....	28	.....	1304	.....	3748	.....
3	633	438	505	544	1333	682	63	22	1006	612	3540	2298
8	574	258	836	416	1341	480	252	51	725	15	3728	1220
15	633	94	484	107	702	81	851	2	58	0	2728	284
20	611	18	321	17	404	0	675	0	270	0	2281	35

* C, control; T, treated.

treated plants during the experiment. A decrease in the amount of these constituents in the untreated plants was to be
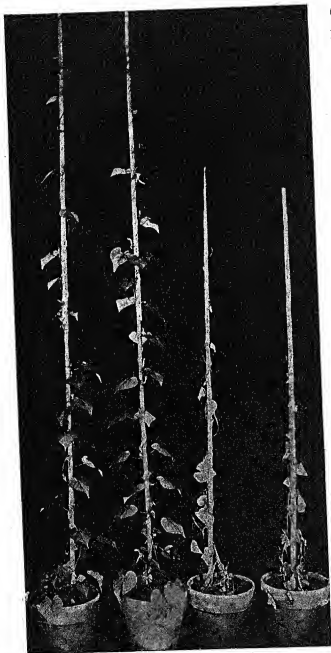
FIG. 2.—Morning-glory plants exposed to 12- to 13-hour photoperiod and grown in relatively fertile soil to hasten vegetative growth and seed production. Two untreated plants (left) compared with two plants sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (right). Twenty-one days after treatment.

expected, since both sets of plants were subjected to adverse conditions of photoperiod and soil fertility.

VEGETATIVE PLANTS

METHODS.—Morning-glory plants were grown under greenhouse conditions in heavily composted soil contained in 6-inch clay pots. On April 8, approximately 600 plants were selected for size and uniformity and arranged on two greenhouse benches. Alternating rows of these plants were then sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. The remaining plants were left untreated. In order to maintain vigorous vegetative growth, the soil in which the plants were growing was treated with a solution containing 2000 p.p.m. of calcium nitrate during the first week following treatment and the surface of the soil in each pot was covered with well-rotted manure.

Thirty treated and thirty untreated plants were harvested at intervals during the 20-day period immediately following treatment. They were divided into roots, hypocotyls, stems, flower buds, and leaves. These samples were then dried at 80° F., ground, and analyzed for total reducing sugars and for starch and dextrin (6).

RESULTS.—The sprayed plants showed marked epinasty within 24 hours after treatment, but the leaves remained green until approximately the sixth day, when most of those on the treated plants changed from green to red and yellow and later to a brownish color. The sprayed plants failed to grow in length following treatment, while an average increase of approximately three expanded internodes was recorded for the untreated plants. The total average dry weight of the sprayed plants decreased slightly during the 3-week period following treatment, while that of the untreated plants increased by 175% during

the same period. Plants sprayed with the mixture containing 1000 p.p.m. of the acid did not exhibit swelling of tissues or external evidence of gall formation, as has been reported in connection with the use of equal concentrations of this acid on other species of plants (4).

The development of buds on treated plants was noticeably inhibited during

plants decreased from 18.4% to 2.1% (dry-weight basis), while that of controls increased slightly during the 3-week period immediately following treatment (fig. 3). Sugar at first accumulated in the treated plants, and on the sixth day they contained approximately twice as much as did the untreated ones. After 3 weeks, however, the treated plants contained
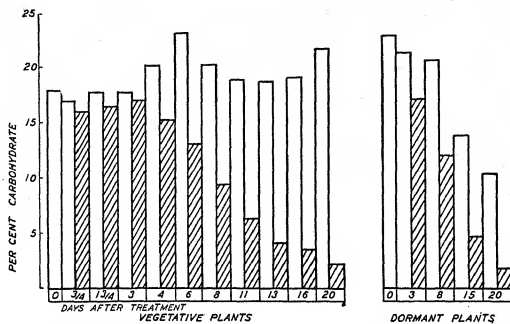
FIG. 3.—Percentage of carbohydrates (sugar, starch, and dextrin) in vegetatively vigorous and in relatively dormant morning-glory plants treated with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (shaded bars), compared with that in untreated plants (unshaded). Values represent carbohydrate contained in samples from thirty plants collected at successive intervals following treatment.

the first 3 days following treatment. On the fourth day untreated plants had an average of three open flowers per plant, while no flowers had opened on the sprayed ones on this date. Two weeks after treatment, 35% of the roots and all the leaves of the treated plants were dead. Decayed areas had developed at the ground level on stems of nearly all the treated plants. Three weeks after treatment the stems, buds, leaves, and 95% of the roots of the sprayed plants were dead (fig. 2).

The readily available carbohydrates (sugars, starch, and dextrin) in sprayed

only 15.9% as much sugar as did the unsprayed.

The greatest increase in sugar content as the result of treatment occurred in the leaves. They contained three to eight times as much sugar as did the leaves of unsprayed plants during the 4–8 days following treatment. In contrast, the sugar content of the flower buds of treated plants did not exceed that of buds on the unsprayed plants, but instead the amount in the treated ones decreased rapidly during the 2 weeks immediately following treatment, and at the end of this period they contained only a trace

of sugar (table 3). Only 18 hours after treatment the flower buds of the treated plants contained approximately 46% less sugar than did the buds of untreated plants.

flower buds of treated plants contained only a trace of reserve carbohydrates in the form of starch and dextrin. Depletion of starch and dextrin reserves in most parts of the plants was clearly evident

TABLE 3

TOTAL SUGAR AND STARCH-DEXTRIN CONTENTS OF MORNING-GLORY PLANTS GROWN IN GREEN-HOUSE UNDER CONDITIONS FAVORABLE FOR VIGOROUS VEGETATIVE GROWTH AND SPRAYED WITH AQUEOUS MIXTURE CONTAINING 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. FIGURES REPRESENT MILLIGRAMS OF CARBOHYDRATES IN THIRTY PLANTS

Days	Roots		Hypocotyls		Stems		Buds		Leaves		Total	
	C*	T	C	T	C	T	C	T	C	T	C	T
							Sugar					
0	324	......	52	......	431	......	36	......	260	......	1103	......
¾	246	183	39	33	475	537	54	29	352	374	1166	1156
1¼	267	324	39	44	475	572	84	30	280	648	1145	1618
3	304	266	40	58	412	532	77	17	295	681	1128	1554
4	341	225	55	64	487	519	88	20	243	766	1214	1594
6	462	192	53	78	390	675	51	25	148	1222	1104	2192
8	247	107	48	74	389	561	164	18	225	789	1073	1549
11	453	59	60	52	440	274	222	4	190	613	1365	1002
13	389	28	113	32	280	175	159	1	120	430	1061	666
16	52	19	52	50	446	224	369	11	238	314	1157	618
20	758	38	100	32	758	146	739	10	1008	310	3363	536
							Starch-dextrin					
0	400	......	222	......	749	......	37	......	2917	......	4325	......
¾	429	393	275	242	894	917	40	19	2645	2163	4283	3734
1¼	570	434	300	258	1325	1226	45	12	2553	2613	4793	4543
3	774	391	430	267	1947	875	64	7	2830	1887	6045	3427
4	807	437	401	253	2375	704	68	1	2970	1354	6621	2749
6	1072	240	635	237	4030	897	109	10	4494	936	10340	2320
8	1093	182	640	168	4279	486	172	1	2492	436	8676	1273
11	1475	161	655	116	5057	435	396	4	1536	259	9119	975
13	1743	120	443	81	5328	210	489	5	1618	81	9621	497
16	1698	73	640	70	5711	116	723	1	2357	11	11129	271
20	2882	21	633	6	6478	0	1393	0	3108	0	14494	27

* C, control; T, treated.

The starch and dextrin content of treated plants decreased steadily from the fourth day following treatment, and on the twentieth day the sprayed plants contained approximately one five-hundredth as much starch and dextrin by weight as did the untreated ones.

Four days following treatment the

on the third day following treatment (table 3).

During the third week after treatment a marked increase in the weight of the untreated plants occurred which was associated with a corresponding increase in their carbohydrate content. This flush of growth on the part of unsprayed plants

was probably due to increased soil fertility resulting from the application of manure during the first week following treatment.

Discussion

Responses of morning-glory plants which were vigorously growing and sprayed with 2,4-dichlorophenoxyacetic acid were similar to those of other plants those in the roots and flower buds, were rapidly depleted following treatment (figs. 3, 4). Depletion of the carbohydrates was apparently a factor which resulted in death of the plants. It has been reported (5, 6) that starch and dextrin were depleted in bean leaves treated with naphthaleneacetic acid or certain other growth-regulating substances, and that sugar accumulated more slowly in illuminated
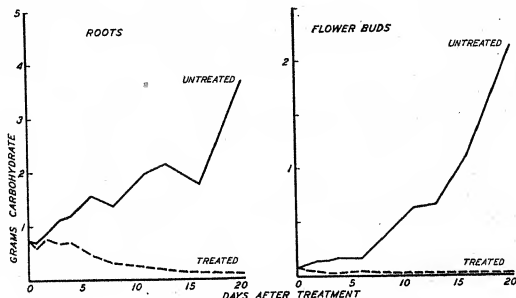
Fig. 4.—Readily available carbohydrate content (sugar, starch, and dextrin) in roots and buds of morning-glory plants during 3-week period following treatment with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500, compared with that of untreated plants. Plants grown under conditions favoring vigorous growth. Thirty treated and thirty untreated plants sampled at each period.

that were in a relatively dormant condition when treated with the acid. Treatment inhibited both vegetative growth and seed development, and when measured in terms of length of time required for death to occur, the effectiveness of this acid was approximately the same on both the actively growing and the relatively dormant plants.

The sugar content of sprayed plants first increased above that of untreated plants, then decreased, while the starch-dextrin reserves were rapidly depleted as the result of treatment. The readily available carbohydrate reserves, especially leaves that had been depleted of reserve carbohydrates and then treated than it did in comparable unsprayed ones. In these earlier experiments photosynthesis was apparently inhibited by the application of naphthaleneacetic acid. It is possible that the synthesis of carbohydrates by the morning-glory plants of the present experiment was likewise impaired by treatment with the 2,4-dichlorophenoxyacetic acid.

In sprayed plants, necrotic tissues first appeared in the flower buds. This response was also associated with a marked decrease in the carbohydrate content of

the buds and indicated that the reproductive parts of the plants were especially sensitive to the acid treatment. Stems of the treated plants were attacked by soil organisms in the region near the surface of the soil, and the necrotic areas that developed as a result may have also been associated with death of the plants.

The marked changes observed in the relative amounts of the carbohydrate content of the plants treated with 2,4-dichlorophenoxyacetic acid suggested the possibility of utilizing this type of response in controlling the rate of hydrolysis of polysaccharides in other kinds of plants, in their seeds and fruit.

Summary

1. Plants of annual morning-glory, *Ipomoea lacunosa* L., grown in a greenhouse under conditions favoring vigorous vegetative growth and seed production, were sprayed with an aqueous mixture containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid and 0. 6% Carbowax 1500. Other plants of morning-glory grown under conditions of photoperiod and soil fertility less favorable for vegetative growth were treated in a similar way.

2. No apparent growth occurred, either in those treated plants that were in a vigorous vegetative condition or in those that were relatively dormant at the time of treatment. Following treatment, the total dry weight of untreated plants increased while that of the sprayed ones decreased. Neither those that were vegetative nor the dormant ones showed external evidence of gall formation or root initiation as the result of treatment. In the sprayed plants, necrosis was first evident in the flower buds, and none of these developed mature seeds. Both the vegetative and the nonvegetative plants died within 3 weeks following treatment.

3. Readily available carbohydrates (sugars, starch, and dextrin) were essentially depleted within a period of 3 weeks in plants that were growing vigorously, and also in plants that were relatively dormant when treated. Carbohydrate reserves (starch and dextrin) were rapidly depleted in the flower buds, and also in the roots, of the sprayed plants, a response of significance in connection with the use of 2,4-dichlorophenoxyacetic acid in the control of weeds.

4. Sugars in treated plants at first increased above the amount in the untreated ones, then decreased, and they were nearly depleted during the second and third week after treatment.

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HERBICIDAL PROPERTIES OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN DUSTS CONTAINING HYGROSCOPIC AGENTS

PAUL C. MARTH,[1] F. F. DAVIS,[2] AND JOHN W. MITCHELL[3]

Introduction

Interest in the dust method of applying growth-regulating substances to plants has been manifest because of the rapidity, uniformity, and relatively low costs of application. In some instances the results obtained have compared favorably with those secured through the use of comparable amounts applied in aqueous solutions (1, 2).

In the present experiments, 2,4-dichlorophenoxyacetic acid was applied to various species of weeds grown under greenhouse conditions to determine the effectiveness of the acid as a selective herbicide (a) when used with various kinds of dust carriers, and (b) when applied with a mixture of carrier and certain water-absorbing agents.

Preparation of dust mixtures

Finely ground Pyrax, fuller's earth, "Cherokee Clay," and purified talc were tested as carriers. To prepare the various

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dust mixtures, the desired amount of 2,4-dichlorophenoxyacetic acid was first dissolved in 95% grain alcohol of sufficient volume to produce a thin paste when stirred into a weighed amount of the carrier (approximately 1 ml. of alcoholic solution per gram of dust). After stirring, the paste mixture was placed in a well-ventilated oven at 80° C. and the alcohol evaporated. When dry, the preparation was thoroughly repulverized while warm and then either stored in airtight containers or used immediately. In preparing dust mixtures with such hygroscopic agents as Carbowax 1500 or glycerin, the same procedure was followed except that the growth-regulating substance was first dissolved in the required amount of hygroscopic agent and then this solution was added to the alcohol.

In preliminary experiments it was found that 3.0% (by weight) of either Carbowax 1500 or glycerin in the carrier produced a dust that was slightly sticky, but one that could be readily applied. The use of higher concentration of the hygroscopic agents resulted in dusts that were very sticky and difficult to apply.

Calcium chloride used as the hygroscopic agent was finely ground and mixed with the dust in the dry state after the required amount of the acid had been added as just described.

The final dust mixtures were applied in weighed amounts at the rate of approximately 4 pounds per 1000 square feet, or 1.8 gm. per square foot, by means of a small mechanical duster operating at 9 pounds of air pressure.

Both the grass and the clover germinated and grew uniformly, so that the soil surface of the flats was completely covered by the mixture of the two species by February 21, when they were treated. Dust mixtures containing 0.5, 1.0, 2.5, 5.0, and 10.0%, respectively, of 2,4-dichlorophenoxyacetic acid were prepared

TABLE 1

EFFECTS OF VARYING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN DUST FORM TO KENTUCKY BLUEGRASS AND WHITE CLOVER GROWING TOGETHER IN GREENHOUSE FLATS. DUST PREPARATIONS MADE WITH PYRAX AND APPLIED FEBRUARY 21, 1945

CONCENTRATION OF ACID (%)	EXTENT OF INJURY FOLLOWING TREATMENT					
	2 days		15 days		50 days	
	Grass	Clover	Grass	Clover	Grass	Clover
0.5	o*	+	o	+	o	o
1.0	o	+	o	+	o	o
2.5	o	+	o	+++	o	+++
5.0	o	+	++	++++	+	++++
10.0	+++	++	+++	++++	++	++++

* o = No apparent injury.
+ = Slight injury to grass or curling of clover.
++ = Moderate injury to grass or curling of clover.
+++ = Severe injury to grass or curling of clover.
++++ = Killing of clover (85-100% dead).

Investigation

PYRAX, FULLER'S EARTH, AND CHEROKEE CLAY AS CARRIERS

KENTUCKY BLUEGRASS, WHITE CLOVER.—On January 6, 1945, each of forty-eight flats (12 × 26 inches) containing fertile potting soil was sown uniformly with 2.83 gm. Kentucky bluegrass and 0.70 gm. white clover, rates approximately equivalent to 3 lb. and ¾ lb. of seed per 1000 square feet, respectively. After seeding, the flats were maintained in a greenhouse at approximately 60° F.

with each of the carriers—Pyrax, fuller's earth, and Cherokee Clay.

Each of the forty-eight flats was divided crosswise into three plots of equal size, and each of the forty-five dust mixtures was applied to three plots at random, leaving three randomized plots as untreated controls. Immediately prior to dusting, all plots were exposed to a fine aqueous mist, so that moisture was deposited on the foliage to simulate a heavy dew. Records of the effect of treatment on the grass and clover were obtained at the end of 2, 15, and 50 days following treatment.

The data on the Pyrax dust plus the acid treatments only are presented in table 1, since the results with fuller's

earth and Cherokee Clay were of the same order. Only the relatively high concentrations of the acid (2.5–10%) effectively controlled the clover. Both the 0.5 and the 1.0% concentrations were ineffective, and at the end of 50 days the clover had recovered completely from the initial curling effects that were apparent at the end of 2 days. Dust applications containing 2.5% concentration of the acid were without serious effect on the bluegrass but greatly reduced the growth of the clover. An estimated 15% of clover remained in these plots at the end of 50 days following treatment. The use of dust with 5.0% of the acid resulted in a moderate amount of tip-burn on the bluegrass foliage but apparently did not greatly retard its development. A dense stand of grass developed in these plots, while the clover was almost completely killed (99%). The bluegrass developed considerable foliage injury when treated with dusts containing 10.0% of the acid. In these treatments the grass had not recovered at the end of 50 days, and only a thin stand was growing at that time (fig. 1). In contrast, all the white clover was killed by the 10.0% acid-dust treatments. No marked differences in behavior of bluegrass or clover were observed in comparing the three carriers at the various concentrations of acid used. From this standpoint it would appear that each of these materials would be satisfactory as a carrier for this acid.

HEAL-ALL, HYDROCOTYLE, AND PLANTAIN.—A 10.0% concentration of 2,4-dichlorophenoxyacetic acid in Pyrax was applied to pure stands of heal-all (*Prunella vulgaris*) and hydrocotyle (*Hydrocotyle rotundifolia*) growing in flats, and also to narrow-leaved plantain (*Plantago lanceolata*) established in potting soil contained in 3-inch clay pots. These plants were maintained in the same greenhouse

section at 60° F. and treated on February 21 in a manner and rate similar to that described for the bluegrass-clover experiment. At the time of treatment the foliage of the heal-all and hydrocotyle covered most of the soil surface of each flat. Three areas, consisting of one third of the flat each, were treated in the case of heal-all and hydrocotyle, leaving three untreated areas of equal size. Fifteen each of treated and untreated plantain plants were used.

The 10.0% acid dust proved to be toxic to all three species. The hydrocotyle and heal-all were severly curled within 48 hours after treatment. After 15 days all dust-treated plants of these two species were dead, while the narrow-leaved plantains were severely curled and chlorotic at this time and were dead a month later.

ADDITION OF WATER-ABSORBING AGENTS TO ACID-TALC DUSTS

PRELIMINARY TESTS.—Several hygroscopic substances were tested for their relative merits as moisture-absorbing agents. A weighed amount of each compound was placed for a period of 164 hours in a closed vessel and exposed to air in which the relative humidity was maintained at approximately 79.5% by means of a saturated solution of ammonium chloride. The percentage increase in weight was 130.9 for calcium chloride (anhydrous), 43.9 for copper sulphate, 40.5 for glycerin, 22.3 for Drierite, 28.2 for Carbowax 1500, 25.0 for Carbowax 400, 17.9 for Carbowax 1540, and 11.1 for pumpkin-fruit tissue (dried and powdered). Of these substances, calcium chloride, Carbowax 1500, and glycerin were selected for use in the dust experiments. The use of polyethylene glycols (Carbowax) in applying growth substances to plants has recently been described (3).
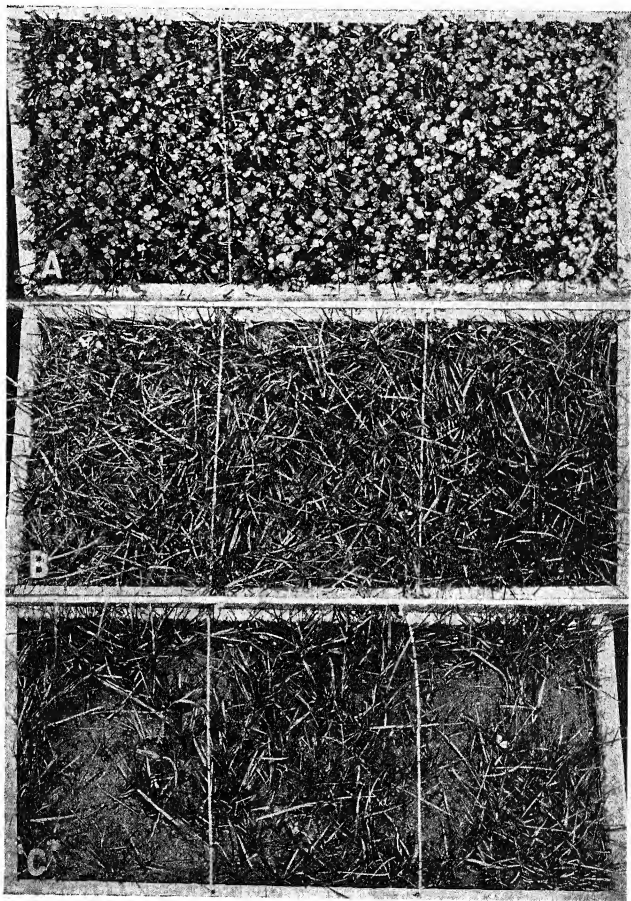
Fig. 1.—Effect of dusts containing 2,4-dichlorophenoxyacetic acid on eradication of white clover from Kentucky bluegrass growing in the greenhouse: *A*, control plots; *B*, plots treated with 5.0% of the acid applied in three dust carriers (left, Pyrax; middle, fuller's earth; right, Cherokee Clay); *C*, plots treated with dusts containing 10.0% of the acid (left, Pyrax; middle, Cherokee Clay; right, fuller's earth). Note excellent control of clover in *B* and *C* as well as poor stand of bluegrass in *C*. Treated February 21; photographed April 13, 1945.

MORNING-GLORY.—Plants of *Ipomea lacunosa* were grown in a greenhouse from seed sown on January 25, in potting soil contained in 3-inch clay pots. A temperature of 65°–70° F. was maintained. After the seedlings had developed the first pair of true leaves, the plants in 600 pots were thinned to two per pot. In the first experiment (March 7), dust treatments (table 2) were applied to 400 plants of this lot having six leaves, and the same treatments were applied again

dust form was markedly increased by inclusion of a moisture-absorbing agent in the dusts (table 2). This effect was most noticeable at the lower concentrations used—0.01 and 0.05%. At the 0.01% concentration the regular acid dust had no apparent effect on the plants, whereas those plants treated with dusts containing 0.01% of the acid to which 3% of either glycerin or Carbowax 1500 had been added showed marked epinasty and stem bending at the end of 48 hours,

TABLE 2

RESPONSE OF MORNING-GLORY PLANTS TO APPLICATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN TALC-DUST MIXTURES CONTAINING MOISTURE ABSORBING AGENTS. RESULTS OF THREE GREENHOUSE EXPERIMENTS (MARCH 7, MARCH 15, AND APRIL 30, 1945) COMBINED

CONCENTRATION OF ACID (%)	NO HYGROSCOPIC AGENT ADDED TO DUST		CARBOWAX 1500 (3%) ADDED TO DUST		GLYCERIN (3%) ADDED TO DUST		CALCIUM CHLORIDE (3%) ADDED TO DUST	
	48 hours	4 weeks	48 hours	4 weeks	48 hours	4 weeks	48 hours	4 weeks
0.........	o*	o	o	o	o	o	o	o
0.01.......	o	o	+	++	+	++	+	o
0.05.......	+	o	++	++++	+	+++	+	+
0.1........	+	+	+++	++++	++	+++	+	++
0.5........	++	++++	+++	++++	+++	++++	++	++++

```
    *  o = No apparent effect.
       + = Slight bending but plants continued linear growth.
      ++ = Moderate bending with sharp checking of linear growth.
     +++ = Severe bending with complete stoppage of linear growth.
    ++++ = All plants dead.
```

1 week later (March 15) to plants having eight leaves. In both experiments, twenty plants (ten pots) per treatment were used. In a third experiment the same treatments were applied on May 28 to another set of morning-glory plants that had developed four to five leaves. These plants were selected from 300 pots sown on April 30. Only plants of marked uniformity of size and appearance were used, and the dust applications were made to the dry foliage at the rate previously stated.

Uniform results were obtained in the three experiments with morning-glory. The effectiveness of the acid applied in

and these plants failed to grow an appreciable amount during the 4-week period following treatment. In contrast, the treated plants that received the same amount of the acid, but with calcium chloride added, showed slight bending but recovered and produced approximately the same amount of vegetative growth as did the untreated plants during the 4 weeks following treatment.

Differential effects between the three hygroscopic agents were most noticeable with dusts containing 0.05% of the acid. At this concentration and without hygroscopic agents added the plants recovered from the effects of treatment.

Addition of 3% Carbowax 1500 to the dust resulted in the death of all the treated plants after 4 weeks, while plants that received the same amount of the acid but with glycerin added were injured severely and apparently unable to recover, although some were still alive at the end of this period.

Calcium chloride added to the dusts did not inhibit growth as much as did either the Carbowax or glycerin mixtures, even though calcium chloride itself was by far the most effective moisture-absorbing agent of the three compounds tested. The ineffectiveness of the chloride dust may possibly be due to differences in its physical properties in relation to cohesion to the plants, or possibly to chemical reactions with the acid.

In these experiments with morning-glory, greatest differences in herbicidal effects of the several dust mixtures were noted at 0.5% concentration. The greatest initial response at this concentration was found in plants treated with dusts containing either Carbowax or glycerin. All plants treated with any dust mixture containing 0.5% acid died within 4 weeks following treatment, and at this concentration and lapse of time the effect of adding a water-absorbing agent was no longer apparent.

RAGWEED.—Plants of *Ambrosia bidentata* growing in 3-inch pots in fertile soil were treated with the same dust mixtures and at the same rates as described for the morning-glory tests of March 15. When dusted, the plants were 4–5 inches in height and were growing vigorously. Ten plants were included in each of the treatments.

Ragweed failed to show a noticeable effect when treated with dust containing 0.1% or less of the acid, while at a 0.5% concentration inhibition of growth occurred only in the case of those dusts

containing 3% of either Carbowax 1500 or glycerin. The plants in these last-mentioned lots, however, were not killed by treatment at the end of 4 weeks, although they had made no apparent growth during this period.

LAMB'S-QUARTERS.—Ten plants each of *Chenopodium album* growing in 3-inch pots were included with the April 30 dusting treatments on morning-glory. The plants were 6–7 inches in height and growing vigorously when treated.

Inclusion of a moisture-absorbing agent increased the effect of the acid on the plants (fig. 2). Dusts containing 0.1% or less of concentration had no apparent effect, except in the case of a few plants which later recovered completely. The plants of lamb's-quarter were killed by applications of dust containing 0.5% of the acid and 3% of either Carbowax 1500 or glycerin.

Discussion

It is evident that 2,4-dichlorophenoxyacetic acid is relatively active when applied in dust carriers, especially when a water-absorbing agent is added to the mixture. On the basis of the present experiments, somewhat larger amounts of the acid were required to bring about a given response when applied in dust carriers than were required for equal effects with liquid sprays (4).

A factor which may restrict to some extent the use of growth regulators in dust carriers is the difficulty of confining the dust treatments within the necessary area. This difficulty may be particularly serious in connection with the use of the acid as a herbicide. In eradicating weeds, the accidental treatment of sensitive crop plants would be more likely to occur during the application of dust treatments than during the use of sprays.

Treatment of large areas of turf, grass-

lands, or cultivated grass crops with dusts for the purpose of selective eradication of certain weeds appears to be feasible, but further experimental work is needed to determine the usefulness of this method of application under a variety of field conditions. The results reported here with the various acid-dust mixtures were with application to plants in a greenhouse, and no attempt was made to simulate intermittent dews, rains, and other weathering conditions commonly found in the field. These conditions might modify results with the various combinations used in the work reported here.

Summary

1. 2,4-Dichlorophenoxyacetic acid was applied in different concentrations (0.5,
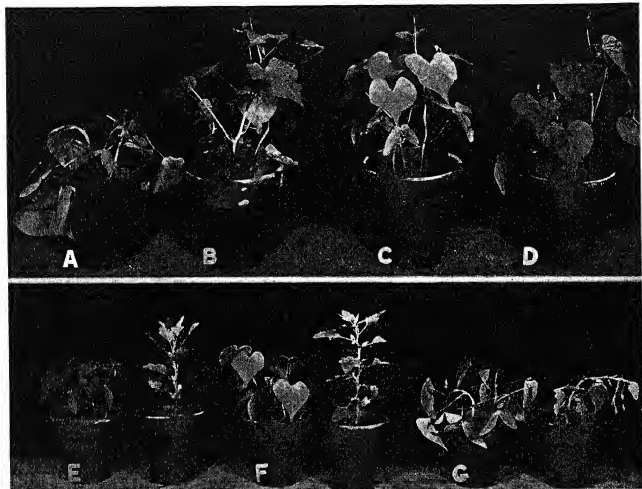
FIG. 2.—Effect of addition of hygroscopic agents to talc-dust mixtures containing 2,4-dichlorophenoxyacetic acid: *A*, morning-glory (*Ipomea lacunosa*) plants treated with talc containing 0.1% of acid plus 3.0% of Carbowax 1500 as hygroscopic agent. *B*, similar plants treated with dust containing same amount of acid but without hygroscopic agent. *C*, plants treated with talc dust containing 3.0% of Carbowax only. *D*, untreated control. *E*, morning-glory at left, lamb's-quarters (*Chenopodium album*) at right, untreated control plants. *F*, similar plants treated with talc dust containing 0.5% acid only. *G*, comparable plants that received dust containing 0.5% acid plus 3.0% glycerin as hygroscopic agent. Photographs taken 3 days after treatment (April 30, 1945). Plants in treatments *A* and *G* were dead 28 days following treatment, while plants in all other treatments were apparently unaffected.

ported here with the various acid-dust mixtures were with application to plants in a greenhouse, and no attempt was made to simulate intermittent dews, rains, and other weathering conditions commonly found in the field. These con-

1.0, 2.5, 5.0, and 10.0%) in dust carriers, at the rate of 4 pounds per 1000 square feet, to Kentucky bluegrass (*Poa pratensis*) and white clover (*Trifolium repens*) growing in a mixed stand in greenhouse flats. The 10.0% dusts were also tested

on heal-all (*Prunella vulgaris*) and lawn pennywort (*Hydrocotyle rotundifolia*) in flats and on narrow-leaved plantain (*Plantago lanceolata*) growing in 3-inch clay pots in a greenhouse.

2. Comparison of Pyrax, fuller's earth, and Cherokee Clay as diluents or dust carriers at each of the concentrations indicated that there was no marked difference among them on the basis of the plant responses observed.

3. Complete eradication of clover from bluegrass turf was obtained by applications of dust containing 10.0% of the acid, but the bluegrass was severely injured and recovered only slowly. Almost complete eradication (99%) was obtained by application of dust containing 5.0% of the acid, and the grass recovered quickly from the slight injury that developed. A minimum concentration of 2.5% of the acid was necessary to kill an appreciable amount (85%) of clover. No bluegrass injury was observed at this concentration.

4. Heal-all, lawn pennywort, and plantain were likewise killed by application of dusts containing 10% of the acid.

5. Addition of hygroscopic agents, such as Carbowax 1500 or glycerin, at the rate of 3% markedly increased the effects of the growth-regulating substance in dust mixtures. Addition of calcium chloride to the regular dusts resulted in only a slight increase in effectiveness of the acid. Plants of morning-glory (*Ipomea lacunosa*) were killed by dusts containing 0.05% concentration of acid plus 3% Carbowax 1500, while comparable plants were but slightly affected and recovered from treatment with the same kind of dust having an equal concentration but no hygroscopic agent added.

6. Plants of ragweed (*Ambrosia bidentata*) were not evidently affected by the application of dusts containing 0.5% of the acid. Comparable plants exhibited severe epinasty and failed to grow following treatment with a 0.5% acid dust to which either Carbowax 1500 or glycerin had been added.

7. Similar results were obtained with plants of lamb's-quarters (*Chenopodium album*), which were killed after 4 weeks by application of dust containing 0.5% of acid and 3% of Carbowax 1500 or dust containing the same amount of the acid but with glycerin added. The plants were but slightly affected by comparable dusts that did not include the hygroscopic agents.

8. Caution should be exercised in applying 2,4-dichlorophenoxyacetic acid by the dust method, first to avoid accidental treatment of adjacent plants by drifting of the dust particles, and second to avoid breathing the dust mixtures during application until definite information is available on the nontoxicity of the acid.

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CURRENT LITERATURE

Trees and Toadstools. By M. C. RAYNER. London: Faber & Faber, Ltd., 1945. Pp. 71. Illustrated. 6s.

This is a short but lucid account of the interrelationships between certain of the higher fungi and trees. Although primarily designed for the general reader, the book will nevertheless redirect the attention of many botanists to the curious morphological and nutritional features of the associations between higher fungi and trees which the author is so well qualified to describe. "Fungus roots" and the effects of the mycorhiza-forming fungus on the growth of conifers are described and illustrated by photographs.—F. C. STEWARD.

The Structure and Reproduction of the Algae, Vol. II. By F. E. FRITSCH. Cambridge, University Press; New York, Macmillan Co., 1945. Pp. xii+939. $12.00.

The second of a two-volume treatise on the structure and reproduction of the algae has now been published, following the first volume by approximately 10 years. The treatise has been designed to present in the English language a comprehensive account of the morphology of the algae, a task for which the author, who has devoted a fruitful life to this group of plants, is particularly qualified.

FRITSCH has arranged the algae into eleven classes, as follows: Chlorophyceae (Isokontae), Xanthophyceae (Heterokontae), Chrysophyceae, Bacillariophyceae (diatoms), Cryptophyceae, Dinophyceae (Peridinieae), Chloromonadineae, Euglinieae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Cyanophyceae). The first eight of these classes were included in volume I, leaving the last three for volume II.

Throughout both volumes the treatment is essentially morphological, with taxonomic and biologic problems discussed only where they are thought to have a bearing on morphology. The literature of the world has been surveyed and the pertinent facts bearing on the morphology of algae have been carefully and critically assayed and incorporated in both volumes. Essentially all the literature which has either practical or historical value has been included at the end of each section dealing with an order. The citations cover the first half of 1943.

These two volumes should form an essential part of the biological library of every college and university. They are likely to prove equally necessary to all who have a special interest in the algae.—J. M. BEAL.

A Catalog of Illinois Algae. By MAX E. BRITTON. Evanston, Ill.: "Northwestern University Studies in the Biological Sciences," No. 2, 1945. Pp. viii+177. $3.00.

This catalog of the Illinois algae brings together all the algae which have been reported in slightly more than ninety previously published papers. It lists a total of 178 genera and 962 species, varieties, and forms distributed among the following classes: Cyanophyta 29 genera and 98 species, varieties, or forms; Rhodophyta 2–2; Chrysophyta 51–308; Chlorophyta 88–509; Pyrrophyta 3–11; and Euglenophyta 5–34. In a short chapter the literature is reviewed under certain categories—(*a*) taxonomic and floristic studies, (*b*) general ecological and pollination studies, (*c*) studies of algae as animal food supply, (*d*) monographs and other comprehensive reports, and (*e*) miscellaneous papers. For each species, variety, and form listed, the place and date of collection, with the name of the collector, are given. This book should prove highly useful to all those interested in the algae of the midwest region of the United States.—J. M. BEAL.

EFFECTS OF PLANT-GROWTH REGULATORS ON SHOOT DEVELOPMENT AND FIELD SURVIVAL OF FOREST-TREE SEEDLINGS

CARL E. OSTROM[1]

Introduction

The phenomenon of bud inhibition by plant-growth regulators, reported by THIMANN and SKOOG (16), has recently been utilized to bring about a delay in shoot growth of various species of horticultural plants. In general, these studies indicate that dormancy of tops of deciduous trees and shrubs can be prolonged for one to several weeks by spraying the dormant twigs with naphthaleneacetic acid and similar growth regulators in the concentration range of about 0.01–0.1% (6, 8, 10, 12, 13). HITCHCOCK and ZIMMERMAN (6) found that fairly low concentrations (0.02–0.04%) of potassium naphthaleneacetate were effective in delaying the opening of buds of fruit trees if applied during the preceding summer. MARTH (8) prevented premature shoot growth of rose bushes in unrefrigerated storage by exposing the dormant plants to vapors of ethyl and methyl esters of naphthaleneacetic acid in concentrations of 0.3–0.4 mg. per cubic foot of air for 1–16 hours, depending upon the temperature.

In preliminary experiments at the Beltsville Forest Laboratory, MAKI (7) found that leader development of red, shortleaf, and loblolly pines could be arrested for periods of from several months to a full growing season by soaking the

tops of the seedlings for 24 hours in an aqueous solution of 1 gm./l. of indolebutyric acid when the new leaders were several inches long. Leaders of eastern white pine were killed by this treatment.

The use of plant-growth regulators to delay leafing-out of hardwoods and to restrict shoot development of pines appeared to have several potential applications in growing, storing, and planting of forest-tree seedlings. Consequently, a number of applied studies were conducted between October, 1942, and October, 1943, in a survey of the following possible benefits of inhibition of shoot growth: (a) Prevention of excess top growth of coniferous seedlings when they must be retained for an extra year in the nursery; (b) reduction of the ratio of transpiring to absorbing surface to increase the drought resistance of coniferous seedlings; (c) extension of the period of dormancy of shoots in the nursery bed to prolong optimum stage for lifting and planting, and prevention of shoot growth of trees during storage; (d) delay of leaf emergence of trees after planting to reduce transpiration while the roots are becoming established.

The studies comprising this survey are divided for convenience according to (A) nursery-bed, (B) pre-storage, and (C) pre-planting treatments.

To obtain additional information from the same tests, the growth regulators were frequently applied in carriers such as wax emulsion, which were intended to

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serve also as protective foliage coatings. The application of wax emulsions to foliage of trees and shrubs is reported by MILLER, NEILSON, and BANDAMER (9) and by CHADWICK (1) to result in higher survival after transplanting. However, SHIRLEY and MEULI (14) detected no superiority in resistance to artificial drought among pine seedlings sprayed with emulsions of four wax and rubber preparations.

Investigation

A. NURSERY-BED TREATMENTS

1. *Seedlings*

PLAN OF EXPERIMENT.—The object of the nursery-bed treatments was to attempt to find a schedule of application of growth regulators which would partially inhibit shoot growth and thus produce trees of low top-root ratio suitable for planting during the subsequent growing season. The preliminary work by MAKI in 1942 indicated that one application of growth regulator in late spring halted leader development of pine seedlings, but that some species (loblolly and shortleaf pines) renewed leader growth later in the summer. Nine different spraying schedules were tried, therefore, involving both single and repeated sprays at monthly intervals from April through July, 1943, in an attempt to curtail shoot growth partially during the whole season. The trees, furnished by the Maryland Department of Forests and Parks in their College Park Nursery, consisted of one 260-foot bed of 3–0[2] red pine (*Pinus resinosa* Ait.), a similar bed of 2–0 table-mountain pine (*P. pungens* Lamb.), and a 30-foot bed of 1–0 loblolly pine (*P. taeda* L.) (fig. 1).

[2] Age classes of forest planting stock are customarily indicated by two figures, the first of which is the number of years in the seed-bed; the second, the number of years in the transplant bed. In forestry nursery practice, "stock" is a collective term for tree seedlings and transplants.

Treatments applied to red pine and table-mountain pine are shown in table 1. The loblolly pine bed was sufficient for only one trial of each chemical at one concentration. Root pruning was included in the test in order to compare the inhibiting effect of pruning with that produced by growth regulators.

PROCEDURE.—The nursery arrangement provided for two root-pruned and two unpruned bed sections, 4 feet wide and 60 feet long, for each of the two main species. The six combinations of chemical and concentration were randomized among six 10-foot blocks in each 60-foot bed section. The ten spraying schedules shown in table 1 were assigned to ten consecutive segments 1 foot long within each 10-foot block.

Naphthaleneacetic acid and naphthalene acetamide were applied singly in concentrations of 200 and 600 gm./l. A mixture of equal parts of these two substances with equal parts of naphthoxyacetic acid and indolebutyric acid was applied also, in the same total concentrations. Because foresters must deal with many species, mixtures of growth regulators rather than single compounds might eventually be found more dependable for general recommendation.

The growth regulators were all dissolved in water containing 50 gm./l. of lanolin which had been emulsified with 5 gm. of laundry soap. The lanolin concentration was higher than the usual carrier concentration of 5–10 gm./l. The level of lanolin selected was determined by current drought tests in the Shirley-Cass Lake drought machine (15) at the Beltsville Forest Laboratory. These tests showed that resistance of seedlings to artificial drought increased with increasing concentration of lanolin emulsion applied as a protective coating, up to a level of 100 gm./l. or more. However, foliage

injury occurred when the concentration was more than about 70 gm./l. In view of these findings it was desired to test, throughout spring and early summer, the

about 50 cc. per square foot of bed on April 23–24, May 20, June 19, and July 21, 1943. At the time of initial application, the terminal buds had elongated to

Fig. 1.—General view of bed of red pine seedlings sprayed with growth regulators at various times during growing season. Photographed September 9, 1943.

possible injurious effects of one to four applications of the protective coating, at a concentration (50 gm./l.) which was considered safe for foliage application.

The sprays were applied at a rate of

an average length of $\frac{1}{2}$–1 inch, except in loblolly pine, where they were only beginning to swell.

RESULTS.—All the 1-year-old loblolly pines were killed by the sprays in the one

concentration applied (600 mg./l.). The effects of treatment on red pine are shown in table 1.

leaders and needles attained only about two-thirds normal length. The mixture of growth regulators was less effective than

TABLE 1

TOP CONDITION OF RED PINE SEEDLINGS IN NURSERY BED IN SEPTEMBER, 1943, AFTER SPRING
AND SUMMER APPLICATION OF GROWTH REGULATORS

| CHEMICAL | CON-CENTRA-TION (MG./L.) | ROOT PRUNING | BLOCK NO. | MONTH OF SPRAYING | | | | | | | | | CON-TROL |
				April	April May	April May June	April May June July	May	May June	May June July	June	June July	
Mixture*......	200	Unpruned	1	A†	B	F	F	B	F	F	Y₁	Y₂	A
		"	2	A	F	F	F	B	F	F	Y₂	F	A
		Pruned	3	B	B	F	F	C·	F	F	F	F	A
		"	4	B	B	F	F	A	F	F	F	F	A
Mixture.......	600	Unpruned	1	A	D	F	F	D	F	F	Y₁	Y₂	A
		"	2	B	F	F	F	D	E	F	F	F	A
		Pruned	3	A	E	F	F	C	F	F	F	F	A
		"	4	A	E	F	F	E	F	F	F	F	A
Naphthalene acetamide...	200	Unpruned	1	A	B	F	F	B	C	F	Y₁	Y₂	A
		"	2	B	E	F	F	C	E	F	Y₂	F	A
		Pruned	3	B	F	F	F	C	E	F	Y₂	F	A
		"	4	B	E	F	F	B	E	F	F	Y₂	A
Naphthalene acetamide...	600	Unpruned	1	B	F	F	F	D	F	F	Y₁	F	A
		"	2	B	E	F	F	D	D	F	Y₁	F	A
		Pruned	3	B	F	F	F	D	E	F	F	F	A
		"	4	B	F	F	F	D	F	F	Y₁	Y₂	A
Naphthalene-acetic acid...	200	Unpruned	1	A	F	F	F	D	F	F	Y₁	F	A
		"	2	B	F	F	F	D	F	F	Y₂	F	A
		Pruned	3	B	F	F	F	D	F	F	Y₂	F	A
		"	4	B	E	F	F	D	E	F	Y₂	F	A
Naphthalene-acetic acid...	600	Unpruned	1	A	F	F	F	F	F	F	Y₂	F	A
		"	2	B	F	F	F	E	F	F	Y₂	F	A
		Pruned	3	C	F	F	F	F	F	F	Y₂	F	A
		"	4	A	F	F	F	F	F	F	Y₁	F	A

* Equal parts (50 mg./l.) by weight of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, indolebutyric acid.
† A: Normal.
B: Half or more of trees slightly stunted; needles and leaders about two-thirds normal size.
C: Most trees with needles less than one-half normal length; leader length nearly normal.
D: Practically all trees with needles less than one-half normal length; usually some trees dead.
E: Same as D, but about one-half of trees dead.
F: Practically all trees dead.
Y₁: Foliage somewhat yellowed; some trees dead.
Y₂: Foliage severely yellowed; many trees dead.

Applications of naphthalene acetamide and naphthaleneacetic acid in April usually caused over-all stunting of the new growth of red pine seedlings. Both

either of the naphthalene compounds; it resulted in stunting in only five of eight replications.

The response to effective treatments

applied May 20, after needles had partly expanded, consisted mainly in stoppage of needle elongation (fig. 2). There was also a slight thickening of leaders, without much retardation of their growth in length. The solutions which inhibited growth without killing the trees were 200

plied then resulted in yellowing of the foliage and in death of some of the trees.

Killing of the uppermost fascicles of old foliage was general among trees sprayed in May and occasional among trees sprayed in April. This injury was most severe on portions not shaded by
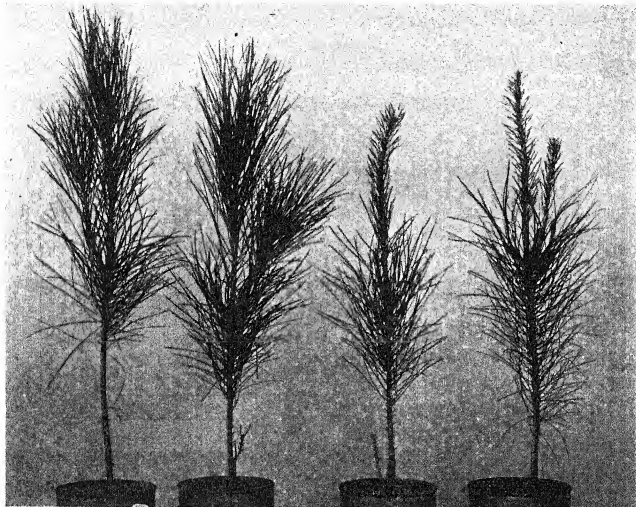
Fig. 2.—Inhibition of needle growth of red pine by two sprayings (April and May) with 600 mg./l. of mixture of growth regulators in 50 gm./l. lanolin. Two untreated controls on left. Photographed July 27, 1943.

mg./l. naphthaleneacetic acid, 600 mg./l. naphthalene acetamide, or 600 mg./l. of the mixture of indolebutyric acid and naphthalene compounds. Naphthaleneacetic acid at this stage was lethal in concentrations of 600 mg.

Single treatments on June 19 had no observable effect on growth of red pine during the current season, for top development was practically complete by that time. However, all treatments ap-

taller seedlings. The injury apparently was due to the lanolin coating where exposed to the summer sun. Although a coating of lanolin from spraying an emulsion of 50 gm./l. usually was not found injurious to conifers planted in the field in the spring, it evidently is injurious to trees in compact nursery beds when applied in hot weather. Cross-sections of red pine needles under a microscope showed penetration of lanolin through

the stomata when emulsions containing 50 gm. or more per liter were sprayed on leaves subjected to a temperature of 96°F.

a concurrent study showed severe injury to transplanted trees by several single applications of lanolin only. However, the growth regulators were partly responsi-

TABLE 2

TOP CONDITION OF TABLE-MOUNTAIN PINE SEEDLINGS IN NURSERY BED IN SEPTEMBER, 1943, AFTER SPRING AND SUMMER APPLICATION OF GROWTH REGULATORS

CHEMICAL	CON-CENTRA-TION (MG./L.)	ROOT PRUNING	BLOCK	MONTH OF SPRAYING									CON-TROL
				April	April May	April May June	April May June July	May	May June	May June July	June	June July	
Mixture*.....	200	Unpruned	1	B†	B	E	E	B	B, E	F	F	F	A
		"	2	A	A	B, E	F	A	C	B	B	Y2, B	A
		Pruned	3	A	B	E	F	A	E	F	Y1, B	F	A
		"	4	A	A	D	F	A	A	B	Y1	Y1	A
Mixture......	600	Unpruned	1	A	E	B	F	C	F	F	Y1	F	A
		"	2	A	E	F	F	C	E	F	Y2, B	F	A
		Pruned	3	B	D	F	F	A, D	F	F	F	F	A
		"	4	A	D	F	F	C	F	F	F	F	A
Naphthalene acetamide..	200	Unpruned	1	A	B	F	B, E	A	B, E	F	Y1	Y2	A
		"	2	A	A	F	F	A	B, E	F	Y1, B	Y1, B	A
		Pruned	3	A	A	E	F	A	E	F	Y1, B	F	A
		"	4	A	A	B	F	A	C	C, E	A	Y2	A
Naphthalene acetamide..	600	Unpruned	1	B	E	F	F	C	F	F	Y1	F	A
		"	2	A	E	F	F	C	E	F	Y1	F	A
		Pruned	3	B	E	F	F	A	F	F	Y1, B	F	A
		"	4	A	C	F	F	A, D	F	F	Y1, B	F	A
Naphthalene-acetic acid..	200	Unpruned	1	A	E	F	F	C	F	F	Y1	Y2	A
		"	2	A	E	E	F	C	F	F	Y1, B	F	A
		Pruned	3	A	D	F	F	A, D	F	F	Y2, B	Y2, B	A
		"	4	A	A, D	E	F	A	E	F	Y1, B	F	A
Naphthalene-acetic acid..	600	Unpruned	1	B, E	F	F	F	D	F	F	Y2	F	A
		"	2	B, E	F	F	F	E	F	F	C, E	F	A
		Pruned	3	A	F	F	F	C	E	F	Y1, B	F	A
		"	4	A	E	F	F	D	F	F	Y2, C	F	A

* Equal parts (50 mg./l.) by weight of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, indolebutyric acid.
† A: Normal.
  B: Half or more of trees slightly stunted; needles and leaders about two-thirds normal size.
  C: Most trees with needles less than one-half normal length; leader length nearly normal.
  D: Practically all trees with needles less than one-half normal length; usually some trees dead.
  E: Same as D, but about one-half of trees dead.
  F: Practically all trees dead.
  Y1: Foliage somewhat yellowed; some trees dead.
  Y2: Foliage severely yellowed; many trees dead.

Two or more applications of the nursery-bed sprays at monthly intervals usually resulted in killing the trees. The injury may have been due to lanolin, for

ble, for the sprays containing the lower concentration of growth regulator were less injurious to the trees (table 1).

Table-mountain pine (table 2) re-

sponded essentially as red pine, with one important difference. The leaders of table-mountain pine became irregularly curved as a result of treatment. At the end of the season these leaders were stiffened into somewhat undesirable shapes. Unless this curving can be avoided by some modification of the treatment, the growth regulators may have no value for those pine species which respond in this fashion. Red pine, noted for its strong apical dominance and straight leader, retained its straightness after treatment.

resistance. Red pines were therefore sprayed in April and May, or in May only (1943), with 600 mg./l. of the mixture of growth regulators and lifted at the end of July of the same season for a test in the Shirley-Cass Lake drought machine. This machine consists essentially of a revolving circular table 6 feet in diameter inclosed in an insulated metal drum 4 feet in height. The plants rest on the revolving table and are exposed to a regulated temperature of 96°F., constant light intensity of about 200 foot-candles,

TABLE 3

SIZE AND WEIGHT OF TREATED AND UNTREATED RED PINE SEEDLINGS, 4 MONTHS AFTER MAY APPLICATION OF SPRAY CONTAINING 600 MG./L. NAPHTHALENE ACETAMIDE

HEIGHT TO BASE OF NEW GROWTH (INCHES)	AVERAGE LENGTH OF NEW LEADER (INCHES)		AVERAGE DRY WEIGHT (GM.)		TOP-ROOT RATIO (DRY WT. BASIS)		BASIS (NO. TREES)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
6. 1–7.0	4.74	5.33	2.94	9.10	3.52	4.10	9	16
7. 1–8.0	5.85	5.91	4.05	10.33	3.87	5.07	6	11
8. 1–9.0	5.84	6.46	5.40	16.00	3.93	4.52	12	5
Average . .	5.48	5.90	4.13	11.81	3.77	4.56	9	11

Measurements of a sample of red pine seedlings at the end of the season showed that treatment of the tops with 600 mg. of naphthalene acetamide per liter of spray in May had resulted in slightly less leader length, a moderately smaller top-root ratio, and a much lower dry weight per tree (table 3).

The lower top-root ratio of treated seedlings was due chiefly to suppression of new foliage by the growth regulators. Treated seedlings had 60% lower root weight and 66% lower top weight than adjacent untreated trees.

It seemed likely that the smaller foliage area and lower top-root ratio resulting from treatment with growth regulators might cause an increase in drought

and wind velocity of several miles per hour. The table is driven by an electric motor at the rate of 6.6 revolutions per minute. Adjustment of a vent in the side of the drum permits humidity to be maintained close to the 20% level, after the initial high rate of transpiration has subsided.

The trees were graded into four size classes, containing twelve trees per treatment, and potted individually in no. 2 tin cans (fig. 2) containing sand adjusted to 5% moisture content. Examinations of the trees in the drought machine were made at 4-hour intervals (except one interval in early morning), and any trees which had reached the point of permanent wilting, as indicated by previous ex-

perience in reviving wilted trees, were removed from the machine. Results of the test, expressed as average survival time, are given in table 4.

An analysis of variance (4) showed that differences between sizes of plants and between treatments were not statistically significant. This test demonstrated that, despite the smaller foliage area of the seedlings with inhibited shoots, they were no more resistant to artificial

TABLE 4

SURVIVAL TIME OF RED PINE SEEDLINGS IN DROUGHT MACHINE, ACCORDING TO SIZE OF PLANT AND TIME OF APPLICATION OF SPRAY*

Size	Average survival time (no. of 4-hour periods)			
	Control	Month of treatment		Mean
		April and May	May	
Large.............	33.2	30.2	29.3	30.9
Medium large.......	31.1	28.8	30.4	30.1
Medium small.......	29.3	28.2	30.3	29.3
Small..............	29.0	22.1	28.0	26.4
Mean (all sizes)....	30.7	27.3	29.5	29.2

* Containing 600 mg./l. of mixture of equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.

drought than were control seedlings at the time the test was made.

In attempts to produce drought-resistant seedlings by inhibition of top growth with growth regulators, it is necessary to consider the possibility that top-pruning also might result in increased drought hardiness. If successful, the latter method would probably be easier for the nurseryman than treatment with chemical sprays. Previous trials of top-pruning of seedlings of ponderosa pine (*Pinus ponderosa* Laws.) by WAHLENBERG (17) before field planting resulted in lower sur-

vival among pruned seedlings than among controls. These findings were checked by the drought-machine technique, using second-year loblolly pine seedlings which had been top-pruned in late spring, 1943. Top-pruned and unpruned seedlings from the same nursery bed were divided into comparable size

TABLE 5

SURVIVAL TIME OF TOP-PRUNED AND UNPRUNED LOBLOLLY PINE SEEDLINGS IN DROUGHT MACHINE

Size	Average survival time (no. 4-hour periods)			
	Degree of pruning			Mean
	Severe	Light	None	
Large..............	22.9	26.3	35.1	28.1
Medium large.......	27.7	33.0	39.4	33.4
Medium small.......	33.2	38.6	41.5	37.8
Small..............	30.1	42.3	29.8	34.1
Mean (all sizes)....	28.5	35.1	36.5	......

MINIMUM SIGNIFICANT DIFFERENCES BETWEEN MEANS:

Criterion	Significant (P-0.05)	Highly significant (P-0.01)
Pruning treatment......	3.2	4.2
Size..................	3.7	4.9
Combination of treatment and size........	6.4	8.4

classes (table 5) and placed in the drought machine on August 18, 1943.

An analysis of variance showed that effects of size class, of degree of pruning, and of the statistical interaction of size and pruning were all significant at the 1% level. Severe top-pruning resulted in a distinct reduction in the resistance of the trees to artificial drought. As in the test of seedlings treated with growth regulators, the smaller foliage area of top-pruned seedlings did not confer

greater drought resistance. The new shoots which developed at or below the point of previous pruning were evidently lacking in drought hardiness, at least at the time of testing.

Some additional top-pruned and unpruned loblolly seedlings were sorted carefully to provide ten trees for each of the size and pruning combinations in table 5. The trees were transplanted in randomized rows in duplicate sand boxes which were watered for a week and then allowed to dry out slowly. After 2 months, survival was 100% for unpruned trees, 72% for lightly pruned trees, and 42% for heavily pruned trees.

These supplementary experiments indicate that top-pruning definitely decreases the drought hardiness of loblolly pine, at least for early fall planting, and support the findings of WAHLENBERG (17) on ponderosa pine.

2. *Transplants*

PLAN OF EXPERIMENT.—To supplement treatments of seedlings with growth regulators in the nursery bed, a smaller test, involving transplants, was made concurrently under different conditions in another nursery at the Beltsville Forest Laboratory. This study included (a) single sprays applied to different trees at various times (semi-monthly) throughout most of the growing season, and (b) comparison of a commercial-wax emulsion with lanolin emulsion as a protective coating and carrier of growth regulators. The test was designed to determine more closely the effect of time of application on response to growth regulators and protective coatings and to show responses in trees transplanted individually rather than in compact nursery beds.

PROCEDURE.—Six hundred 2–0 loblolly pines and 600 3–0 red pines were transplanted April 9 and 14 to parallel

rows, each containing 100 trees spaced 6 inches apart. The following list shows the designation and composition of sprays used:

1. Wax 0 . . . . Commercial-wax emulsion diluted with three parts of water.
2. Wax 200 . . . . . Wax emulsion with 200 mg./l. of the mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.
3. Wax 600 . . . . . Wax emulsion with 600 mg./l. of the mixture of growth regulators.
4. Lanolin 0 . . . . Emulsion of 50 gm. of lanolin and 5 gm. of soap per liter.
5. Lanolin 200 . . . Lanolin emulsion with 200 mg./l. of the mixture of growth regulators.
6. Lanolin 600 . . . Lanolin emulsion with 600 mg./l. of the mixture of growth regulators.

The commercial-wax emulsion, according to MILLER *et al.* (9), contained paraffin wax, bentonite, and a fatty acid emulsifier. Each spray was applied to ten trees of each species at seven semi-monthly intervals from May 14 to August 17, and to six trees on October 4, 1943. In each row, two unsprayed control trees were left between the groups of trees sprayed at successive intervals. To obtain some information on the lethal limit, an additional lanolin emulsion containing 1 gm./l. naphthaleneacetic acid was applied to twelve extra trees of the second spray on May 29.

RESULTS.—Growth regulators exerted a greater inhibiting action on new shoots when applied in wax emulsion than when applied in lanolin emulsion. Seedlings sprayed with growth regulators after elongation of the shoot but before emergence of the needles eventually had fewer needles emerging than control plants. The several sprays varied in effectiveness, from no inhibition by 200 mg./l.

growth regulators in lanolin emulsion to complete inhibition of needle emergence by 600 mg./l. in wax emulsion.
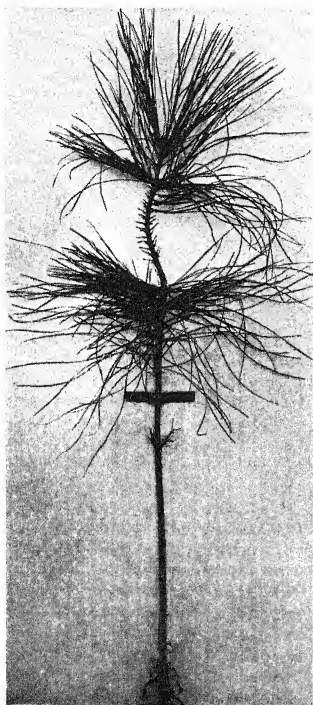
FIG. 3.—Third-year loblolly pine transplant sprayed May 14 with 200 mg./l. of mixture of growth regulators in 1 part commercial-wax emulsion to 3 parts water. Normal growth resumed after initial stunting. Photographed September 23, 1943.

In loblolly pine, inhibition of needle emergence was accompanied by curling of the new shoots, but most of the shoots which were not severely injured had straightened up by the end of the season. Lanolin and wax emulsions without growth regulators had no effect on the emerging leaders.

Growth regulators applied after the beginning of needle emergence checked the expansion of the needles after a few days and resulted in needles of about one-third normal length. A few trees of each species were killed by the sprays containing 600 mg./l. growth regulator. Nearly half of those sprayed with 1 gm./l. naphthaleneacetic acid in lanolin emulsion were killed

The sprays of 200 and 600 mg./l. in lanolin or wax emulsion had no visible effect on red pine when applied after completion of needle elongation, except that all sprays containing lanolin (50 gm. lanolin and 5 gm. soap per liter), when applied on August 4, turned the foliage color of red pine to a yellowish green.

Unlike red pines, loblolly pines normally continue shoot growth through late spring and summer. Treatment of loblolly transplants with growth regulators as late as June, July, or August therefore resulted in modification of subsequent shoot growth. Frequently, when the emergence of needle fascicles was completely prevented by growth regulators, the scale leaf below each fascicle elongated. Shoots of loblolly pine thus modified resembled juvenile leaders which have single needles.

Most of the loblolly seedlings which had been inhibited by growth regulators applied during the early stage of shoot elongation in May resumed normal shoot growth later in the season, as shown by the plant in figure 3. This initial inhibition and subsequent resumption of leader growth would seem at first glance to be advantageous for the survival of planted trees during the first year in the field; top

growth might await the establishment of roots in the new environment, and then proceed normally. Results of field survival studies to test this hypothesis are presented later.

RÉSUMÉ OF RESULTS OF A: NURSERY-BED TREATMENTS

1. Modification of shoot growth of 2- to 3-year-old plants of red, loblolly, and table-mountain pines was brought about by sprays containing 200 mg. naphthaleneacetic acid, 200–600 mg. naphthalene acetamide, or a mixture of several growth regulators per liter of wax or lanolin emulsion.
2. These sprays, when applied just before shoot elongation, usually prevented emergence of most of the new needles but did not prevent the initial surge of leader growth.
3. When applied during the period of needle elongation, the effective sprays arrested needle growth.
4. Inhibited leaders of loblolly and table-mountain pines usually became curved, but red pine leaders remained straight after treatment. Loblolly pines with inhibited leaders resulting from treatment with growth regulators in the spring resumed normal leader growth during the summer.
5. Treated red pines with less than normal foliage area and low top-root ratio were no more resistant than untreated trees to artificial drought during the season in which they were treated. Direct reduction of foliage area of loblolly pine by top-pruning actually decreased resistance of the plants to artificial drought applied several months after pruning.
6. Concentrations of growth regulator which resulted in definite inhibition of the new leaders of pine seedlings usually killed some of the plants. Indications are that one treatment with 600 mg./l. naphthaleneacetic acid is lethal to 1-year-old pine seedlings and that several such sprays will kill older seedlings.

B. PRE-STORAGE TREATMENTS

1. *White ash and loblolly pine*

PLAN OF EXPERIMENT.—Prevention of budding-out of tree seedlings during storage by treatment with growth regulators offers promise of improving survival in the field after late planting. Because no work of this kind had previously been done on species used in forest planting, a rather broad study involving three dates of lifting from the nursery, three types of storage, two dates of planting, and eight growth-regulator treatments was designed in an attempt to define the conditions under which the treatments might be most beneficial in terms of field survival. Advantage was taken of MARTH's previous work (8) in selecting a fairly small number of chemical treatments which could be tried under the various combinations of storage conditions and storage schedules.

The design of the experiment, including all chemical and storage treatments, is shown in table 7. The actual dates of lifting and treating the seedlings were December 1, 1942, and April 15–20, 1943; the dates of planting were May 11–15 and June 16–17, 1943. Half the trees treated and stored in December and half of those treated and stored in April were planted in May, and those remaining were planted in June. All trees treated and stored in May were planted in June. The resulting five periods of storage, applying alike to refrigerated, unrefrigerated, and outdoor conditions, provided fifteen storage treatments, within each of which all the growth-regulator treatments were employed.

PROCEDURE.—The 9000 trees used in this experiment consisted of 1–0 white ash (*Fraxinus americana* L.) and 3–0 loblolly pine. The latter were spindly seedlings of low vigor which later proved extremely susceptible to the shock of transplanting.

Spray solutions were prepared according to MARTH's method (8). Naphthalenemethylacetate and a mixture of naphthalenemethylacetate, naphthalene

acetamide, and naphthoxyacetic acid were employed in total concentrations of 100 and 600 mg./l. in 0.5 % commercial-wax emulsion. These solutions were applied with a pressure sprayer to both tops and roots of the seedlings.

Vapors of the same compounds were applied in a large sterilizer of 233-cubic-foot capacity and a special cylindrical chamber of 20-cubic-foot capacity, with water-sealed lid, at the Plant Industry Station at Beltsville Research Center. The growth regulators, in quantities of 0.3 and 0.5 mg. per cubic foot of chamber space, were mixed with alcohol and volatilized from a hot plate, as described by MARTH. Trees were left in the chambers for 16 hours, at 60°–80°F., with roots packed in sphagnum moss. It was considered necessary for purposes of this study to replace the vapor-saturated with fresh moss before storing the seedlings, in order to reduce the danger of contamination among different treatments during storage.

The common and cold-storage facilities were those at the station at Beltsville. The controlled cold-storage room was maintained at 32°F. and at a relative humidity of 85–90%. In the common-storage shed (unrefrigerated) the temperature fluctuated around 40°F. in winter and rose to as high as 70°F. in the summer. The heeling-in bed, in sandy soil under a pine stand, was heavily mulched with pine litter.

In mid-May and mid-June, seedlings of each species were planted in two series of replicate randomized blocks on a plowed area of sandy soil. Each block contained 150 rows, corresponding to the 150 treatment combinations, with fifteen trees per row. Each block was subdivided into five randomized plots corresponding to the five storage schedules.

Loblolly pines planted in May proved to be in very poor vigor. Those left in storage until June had deteriorated so badly that only trees placed in cold storage in December and in April were saved for late planting. These seedlings from cold storage were utilized in a supplementary test of lanolin foliage coatings, for their poor vigor gave promise that any benefits from the foliage coatings would not be obscured by uniformly high survival. The 579 living loblolly pines available for the coating test were separated into two groups equally divided with respect to the original pre-storage treatments, and trees in one group were dipped from tip to root collar in an emulsion of 50 gm. lanolin per liter of water. The seedlings were planted on June 17, with rows of lanolin-coated and uncoated trees completely randomized.

RESULTS.—Of the loblolly pine trees used in the supplementary test of lanolin coatings, the numbers surviving on different dates are shown in figure 4. After 3 weeks (July 5), mortality was 65% among coated trees and 91% among controls. Thereafter, more rapid mortality of the coated trees brought both groups essentially to equality of survival by the end of the season. The summer of 1943 was extremely dry, with merely 23% of normal precipitation throughout July and August (table 6).

Figure 4 shows that seedlings coated with lanolin emulsion had a definite advantage during the early period of transplanting stress; but the continued drought, affecting more and more unprotected new growth, gradually erased the initial advantage. Final judgment as to whether the initial advantage imparted by lanolin coatings would be maintained through a summer of more nearly normal precipitation must be withheld for the present.

Effects of the growth regulators on

white ash were quite evident when seedlings were removed from storage in May for the first planting (table 7).

Untreated trees from common (unrefrigerated) storage bore numerous etiolated new terminal shoots, some as long as $2\frac{1}{2}$ inches, whereas trees sprayed with 600 mg. growth regulator per liter of wax emulsion bore none. Trees treated

Fig. 4.—Effect of dipping tops of 3-year-old loblolly pine seedlings in emulsion of 50 gm./l. lanolin on seasonal trend of survival in field.

with the low concentration of growth-regulator spray (100 mg./l.) and the high concentration of vapor (0.5 mg./cu. ft.) showed an intermediate stage of shoot inhibition. Of the treated trees exhibiting shoot inhibition, the ones stored in the fall of 1942 usually had a number of short, stout, horizontal roots growing from the lower portion of the stem. Comparably treated trees which had been lifted in April, 1943, and stored for 1 month bore only incipient roots or root swellings along the lower stem. No differences in the small quantity of the normal

type of new root growth could be observed between any of the treatment groups removed from common storage in May.

Seedlings removed in May from storage at $32°$F. were completely dormant. Those which had been treated in the fall and kept over-winter in the heeling-in bed showed no consistent inhibition of top growth, and seedlings treated with the higher concentrations of growth regulator were in slightly more advanced

TABLE 6

Summer Precipitation for 1943 at Belts-ville Forest Laboratory, compared with 40-year average at Laurel, Maryland

Month	Precipitation (inches)	
	1943	40-year average
May...............	3.60	3.48
June...............	2.65	3.99
July...............	1.20	4.26
Aug...............	0.92	4.93
Sept...............	4.25	3.56

stage of leafing. Thus the same treatments with growth regulators applied in the fall caused distinctly greater growth responses in trees stored in a shed than in those heeled-in outdoors. Apparently, when trees are stored outdoors over-winter, treatments to delay shoot growth should be applied in the spring rather than in the fall.

Seedlings lifted and treated in April showed definite inhibition of shoots for a period of 1–2 weeks in the heeling-in bed, and by May many had developed stout new roots below and just above the root collar.

White ash seedlings removed from cold storage for the second (June) planting were completely dormant. The effects produced in common storage by different

treatments were about the same as those previously recorded, except that the etiolated new shoots had become quite long (figs. 5, 6).

As an example of the condition of the trees at the time of the second planting, the detailed effects of the different treatments on one lot kept in common storage from May 13 to June 14 are given in table 8. The effects of the treatments on field performance, which are discussed in the following section, are also given in table 8 for comparison with other effects of treatment.

Seedlings lifted from the heeling-in bed in June for late planting showed much less inhibition of shoot growth than those held in common storage. The sprays containing 600 mg./l. growth regulator delayed leaf emergence for about 2 weeks, but by June 14 normal top growth was present on most of the trees which had been treated while dormant. Roots which developed on the stems of heeled-in trees as a result of treatment were localized just above the root collar, on that portion of the lower stem that had been covered with soil in the heeling-in bed. The high concentration of spray often caused the initiation of stout new rootlets below the collar also (fig. 7). However, the chemical treatments which caused the most pronounced root initiation often injured the trees, as

TABLE 7

RELATIVE EFFECTIVENESS OF GROWTH-REGULATOR TREATMENTS ON WHITE ASH BASED ON DEGREE OF TOP INHIBITION AND ROOT STIMULATION AT TIME OF REMOVAL FROM STORAGE

TYPE OF STORAGE	PERIOD OF STORAGE	CON-TROL	VAPOR TREATMENTS				WAX ONLY	SPRAY TREATMENTS			
			0.3 mg./cu. ft.		0.5 mg./cu. ft.			100 mg./l.		600 mg./l.	
			Nam.*	Mix.†	Nam.	Mix.		Nam.	Mix.	Nam.	Mix.
Cold (32° F.)	Dec.–May.........	....	....	....	....						
	Dec.–June.........	....	....	....	....						
	Apr.–May.........	....	....	....	....	All stock dormant					
	Apr.–June.........	....	....	....	....						
	May–June.........	....	....	....	....						
Common (40°–70° F.)	Dec.–May	o‡	o	o	o	1	o	2	2	3	3
	Dec.–June	o	o	o	o	1	o	2	2	3	3
	Apr.–May.........	1	1	2	1	1	1	2	2	2	2
	Apr.–June.........	o	2	o	·3	o	o	2	3	3	3
	May–June.........	o	2	o	3	2	o	2	3	3	3
Heeled-in outdoors	Dec.–May.........	1	1	1	1	1	1	1	1	o	o
	Dec.–June.........	o	o	o	o	o	o	o	o	o	2
	Apr.–May.........	o	1	o	2	2	o	2	1	3	3
	Apr.–June.........	o	o	o	o	o	o	o	o	2	1
	May–June.........	o	3	o	3	2	o	1	1	3	3

* Nam.: naphthalenemethylacetate.
† Mixture of equal parts of naphthalenemethylacetate, naphthalene acetamide, naphthoxyacetic acid.
‡ o: Active shoot development.
  1: Fairly active shoot development.
  2: Moderate shoot inhibition, often with root initiation.
  3: Severe shoot inhibition, usually with root initiation.

indicated by survival data presented later.

Loblolly pine seedlings failed in general to survive the various storage conditions. Among seedlings heeled-in outdoors over-winter, mortality at the time of removal for the first planting in May was 46.5% for controls and 31.5% for treated seedlings. Although random vari-

of leafing-out of white ash due to the pre-storage treatments (table 9).

The pattern of responses in breaking dormancy in the plantation is in most respects similar to the results discussed in the previous section. The mixture of growth regulators applied in the vapor state actually stimulated budding-out of trees lifted in December (when they were

TABLE 8

EFFECTS OF GROWTH-REGULATOR TREATMENTS ON WHITE ASH LIFTED MAY 13
AND HELD IN SHED STORAGE UNTIL JUNE 14

TREATMENT	LENGTH OF ETIOLATED SHOOTS			DEGREE OF ROOT INITIATION ‡		CONDITION IN PLANTATION OCT. 5		
	None	0"–6"	6"–12"	Above root collar	Below root collar	Good vigor	Poor vigor	Dead
	(No. trees)					(No. trees)		
Vapor method:								
Control..............	0	21	9	0	0	5	12	13
Nam.* 0.3 mg./cu. ft.....	30	0	0	3	0	13	5	12
Mix.† 0.3 mg./cu. ft.....	4	21	5	1	0	13	11	6
Nam. 0.5 mg./cu. ft......	30	0	0	3	3	13	8	9
Mix. 0.5 mg./cu. ft.......	28	2	0	2	2	11	9	10
Spray method:								
Wax only..............	0	18	12	1	0	6	17	7
Nam. 100 mg./l.........	28	2	0	2	3	9	11	10
Mix. 100 mg./l.........	30	0	0	2	3	15	11	4
Nam. 600 mg./l.........	30	0	0	3	3	0	2	28
Mix. 600 mg./l.........	30	0	0	3	3	1	1	28

* Nam.: naphthalenemethylacetate.
† Mixture of equal parts of naphthalenemethylacetate, naphthalene acetamide, naphthoxyacetic acid.
‡ 0: None.
  1: Incipient.
  2: Moderate.
  3: Abundant.

ation was great, a chi-square test of the ratios of living to dead trees showed that the superiority of the survival of chemically treated trees was of medium significance. However, the growth regulators did not prolong the dormancy of buds of loblolly pine, nor did they stimulate root initiation. Further conclusions based on field survival after planting are given in the following section.

Two weeks after the first (May) planting, there were certain differences in rate

relatively resistant to treatment) and kept in cold storage. MARTH (8) also observed stimulation of budding-out by low concentrations of growth regulators in his study of storage of rose bushes. The data in the last line of table 9 summarize the relative effectiveness of the several chemical treatments in prolonging dormancy when applied in the spring before indoor storage. The two spray treatments at a concentration of 600 mg./l. were distinctly injurious; 6 weeks after
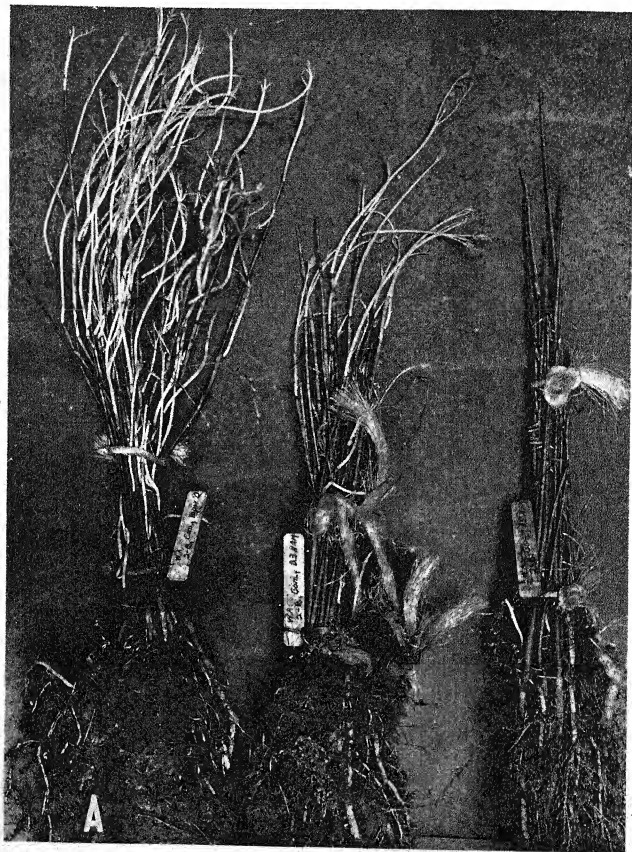
Fig. 5.—Effect of growth regulators on shoot growth and root initiation of white ash seedlings treated April 16 and placed in unrefrigerated storage. *A:* left, untreated; center, exposed 16 hours to vapor of naphthalenemethylacetate (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. *B:* left, untreated; center, exposed 16 hours to vapor of mixture of growth regulators (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. Photographed June 4, 1943.
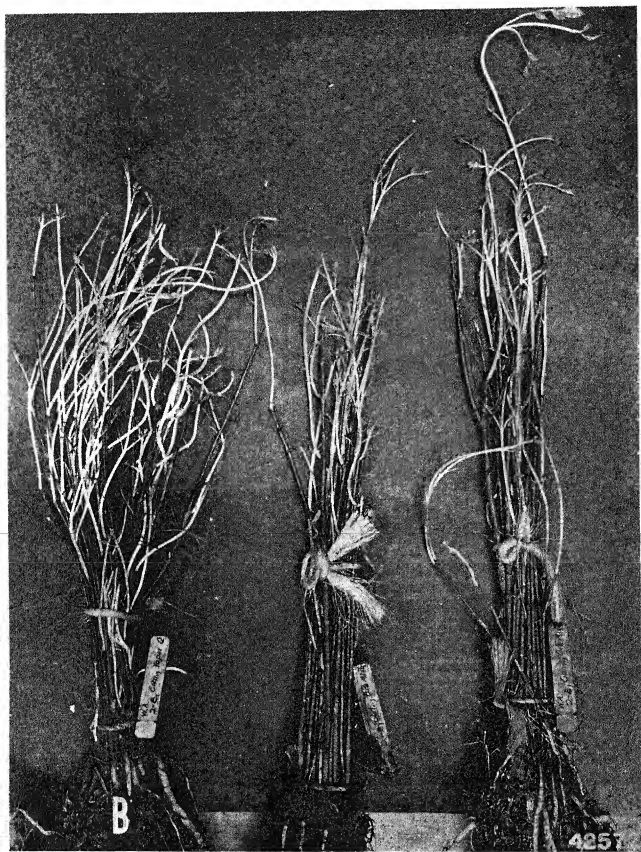
FIG. 5
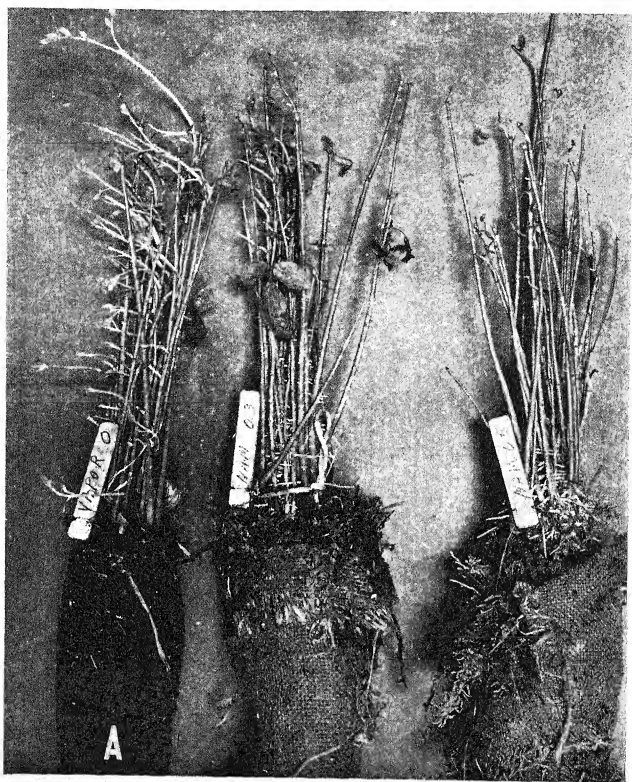
Fig. 6.—Effect of growth regulators on shoot growth and root initiation of white ash seedlings treated May 13 and placed in unrefrigerated storage. *A*: left, untreated; center, exposed 16 hours to vapor of naphthalenemethylacetate (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. *B*: left, sprayed with 0.5% commercial-wax emulsion; center, wax plus 100 mg./l. of mixture of growth regulators; right, wax plus 600 mg./l. of mixture of growth regulators. Photographed June 4, 1943.
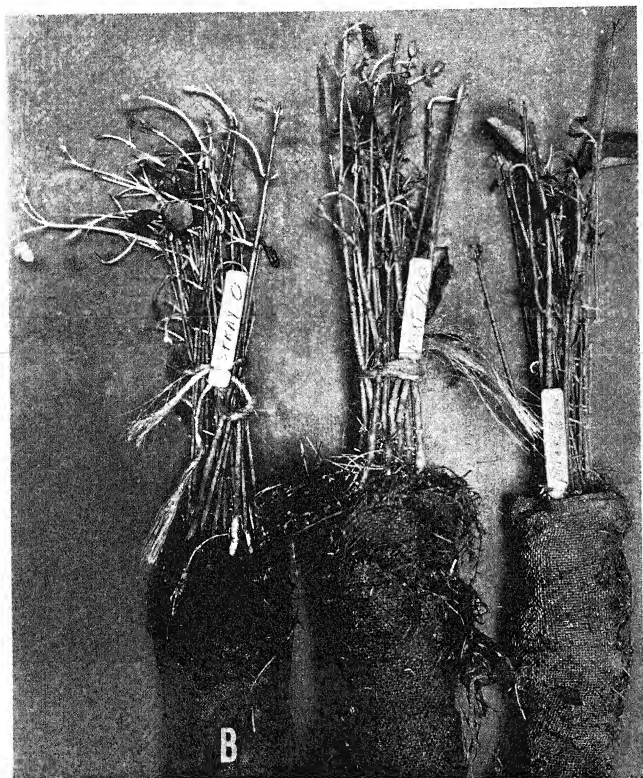
FIG. 6

quence to survival, except that the sprays containing 600 mg./l. growth regulator were definitely harmful.

In the late planting, on the other hand, 20.6% of the control trees died by the end of July, and more severe test of the treatments was obtained. There was an obvious relationship between mortality for different chemical treatments and the effectiveness of the treatments in prolonging dormancy (table 11).

lower than that of trees receiving the weak treatment or no treatment (20.9%).

The superior survival of trees representing the five effective treatments with growth regulators was fairly consistent for all dates of lifting and types of storage (table 11). The relative advantage of the treatments was least among seedlings treated in May and among those stored outdoors. Injury from excessive concentration of growth regulator (600 mg./l.)

TABLE 11

MORTALITY OF WHITE ASH SEEDLINGS JULY 30-31, ACCORDING TO DATES OF LIFT-
ING, TYPES OF STORAGE, AND GROUPS OF CHEMICAL TREATMENTS
(JUNE PLANTING)

TIME OF LIFTING AND TREATING	TYPE OF STORAGE	PERCENTAGE MORTALITY, BY GROUPS OF TREATMENTS					
		Controls (two groups)	Vapor of mixture* (0.3 mg./cu. ft.)	Effective treat-ments†	Conc. sprays (600 mg./l.)	Weighted mean	Basis (no. of trees)
Dec.	All	27.2	26.7	16.7	26.3		900
April	All	15.0	24.4	9.6	52.8	20.8	900
May	All	19.5	13.3	13.3	57.2	23.3	900
All dates	Cold	13.9	8.9	5.1	36.1	13.4	900
All dates	Common	28.3	34.4	18.9	86.6	35.9	900
All dates	Heeled-in	19.5	21.1	15.6	36.7	21.1	900
Mean		20.6	21.5	13.2	53.1	23.5	2700

* Composition of mixture as in table 8.
† Same as for table 10.

An analysis of variance was performed on the July mortality data, omitting the early planting and the two concentrated sprays, which obviously have separate variances. For this analysis, the numbers of dead trees per group of thirty were transformed to their square roots to reduce the right-skewness of the distributions.

The analysis showed that shed storage resulted in significantly greater mortality than other types. For all types of storage combined, the mortality of trees receiving the five "effective" chemical treatments (13.2%) was significantly

was much greater in common storage than in cold storage or heeling-in beds.

The course of mortality through the summer is shown in table 12 for the late planting only and in figure 8 for all trees in the experiment. The gradual closing of the gap in mortality between the control group and the effective treatments very probably represents an influence of the severe drought of 1943 in masking original treatment differences.

An analysis of variance of mortality to October 6 for late-planted trees, excluding the two injurious spray treatments, showed that differences due to

TABLE 12

SEASONAL TREND OF MORTALITY OF WHITE ASH ACCORDING TO GROUPS
OF PRE-STORAGE TREATMENTS; LATE PLANTING ONLY

DATE RECORDED	PERCENTAGE MORTALITY, BY GROUPS OF TREATMENTS				
	Control (two groups)	Vapor of mixture* (0.3 mg./cu. ft.)	Effective treatments†	Conc. sprays (600 mg./l.)	Weighted mean
June 21–23.........	0	0	0.7	16.3	3.6
July 30–31.........	20.6	21.5	13.2	53.1	23.5
Aug. 23–24.........	33.5	31.8	28.3	74.1	28.8
Oct. 5–6...........	35.9	33.7	30.8	75.9	41.1

* Composition of mixture as in table 8.
† Same as for table 10.

growth-regulator treatments were no longer statistically significant. Cold storage was followed by significantly lower mortality (33%) than common storage (51%) or heeling-in (40%).

It was shown previously that control seedlings lifted in December and kept in cold storage over-winter leafed out more slowly than other trees kept in cold storage only in the spring. Comparison of mortality records for these two groups of seedlings revealed no advantage in field survival from the prolonged dormancy which resulted from over-winter storage at 32°F.

Of the white ash seedlings planted in June which survived the first growing season, the proportion in good vigor in October was 47% for controls and 60% for those which had received effective dormancy treatments. On many trees which had been kept until June in common storage, the etiolated storage shoots hardened into crooked shapes after planting. The resulting stem forms of samples of treated and untreated trees are shown in figure 9.

To determine whether growth regulators stimulated root growth after planting, sample rows of trees from one plot planted in June were carefully dug from

the plantation in October. The volume of the root and that of the old stem of each tree were measured by water displacement. The volume of the old stem
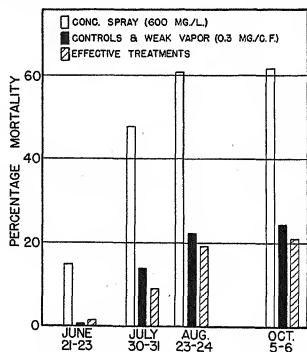
FIG. 8.—Seasonal trend of mortality of white ash seedlings according to groups of pre-storage treatments with growth regulators (May and June plantings combined). "Effective treatments" were those listed below table 13.

(from which all current year's growth had been removed) was considered the most suitable measurement to allow for initial differences in size of plant, and to provide top-root ratio. The data are summarized in table 13.
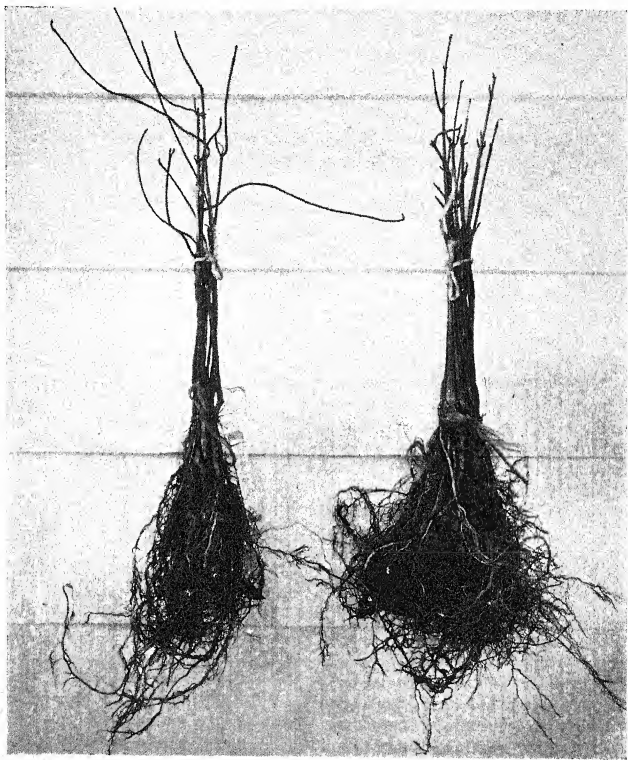
FIG. 9.—White ash seedlings lifted in May, held in common storage 1 month, planted, and then dug up in October, 1943. Left, eleven untreated trees showing crooked form of new shoots originally put forth in storage shed before planting. Right, thirteen trees sprayed with 100 mg./l. of mixture of growth regulators before storage.

The measurements were tested by analysis of covariance (4), using volume of old stem as the independent variable. Variation in top-root ratio among the treatment groups was highly significant, and a further test of the adjusted top-root ratio for seedlings treated with growth regulators against that of the controls showed this difference also to be highly significant. It is concluded that the stimulation of root initiation observed in common storage and in the

lings that had been stored over-winter, these seedlings will be omitted from further consideration. The effect of chemical treatment on the survival of trees lifted and treated in April is shown in table 14.

None of the pre-storage treatments resulted in a consistent increase in survival and most of the chemical treatments were detrimental in this respect. Even seedlings sprayed on tops and roots

TABLE 13

STEM AND ROOT VOLUME OF WHITE ASH SEEDLINGS LIFTED IN APRIL, PLACED IN COMMON STORAGE UNTIL JUNE, AND REMOVED FROM PLANTATION IN OCTOBER, 1943. CHEMICAL TREATMENTS AS IN TABLE 8

| | CONTROLS (TWO GROUPS) | VAPOR TREATMENTS | | | | SPRAY TREATMENTS† | |
| | | 0.3 mg./cu. ft. | | 0.5 mg./cu. ft. | | 100 mg./l. | |
		Nam.	Mix.	Nam.	Mix.	Nam.	Mix.
Av. vol. of old stem (cc.)	2.25	3.40	2.59	2.43	2.80	1.72	2.90
Av. vol. of roots (cc.)	6.32	11.28	8.15	12.84	9.90	7.75	9.67
Av. ratio top/root (vol.)	0.341	0.300	0.286	0.192	0.277	0.213	0.285
Av. ratio after adjustment* . . .	0.333	0.316	0.286	0.191	0.281	0.204	0.291
		0.2615					
Basis (no. of trees)	17	9	13	10	9	8	13

* Adjusted for differences in volume of old stem by analysis of covariance.
† Trees treated with 600 mg./l. spray were nearly all dead.

served in common storage and in the heeling-in beds continued to be effective in the plantation, and that a stimulus to root growth after planting resulted from most if not all of the effective treatments with growth regulators. A review of literature on the use of growth regulators in transplanting, by MITCHELL and RICE (11), indicates that growth of roots of deciduous trees and shrubs was usually stimulated by soaking the roots for various periods in solutions of 10–80 mg./l. indolebutyric acid.

Because mortality in the plantation exceeded 99% among loblolly pine seed-

with wax emulsion containing no growth regulators showed lower survival than controls. Other studies in this series support the conclusions that the commercial-wax spray is detrimental to field survival of conifers when applied to the roots and that this effect is accentuated by the presence of growth regulators.

2. Shortleaf pine

PLAN OF EXPERIMENT.—Because of the great number of species used in forest planting, it was desirable to supplement the main experiments with several smaller tests on other species. One of the

smaller tests of pre-storage treatments involved 1420 3-year seedlings of short-leaf pine (*Pinus echinata* Mill.) which were known to have low resistance to storage and transplanting conditions.

PROCEDURE.—The seedlings were lifted April 15, 1943, for treatment with nine solutions or emulsions of growth regulators (table 15). Lanolin and wax emulsions with and without growth regulators were applied with a 5-gallon hand-sprayer to tops, roots, or whole plants.

RESULTS.—When the seedlings were removed from storage, the proportion in low vigor was 20% for control trees, 21% for trees soaked in growth regulators, 44% for trees receiving root spray, and only 1% for trees receiving sprays of lanolin or commercial wax on tops or tops and roots. The growth regulators had no effect on deterioration in storage. Protective coatings retarded visible deterioration of the seedlings when applied to the foliage but were harmful when ap-

TABLE 14

SURVIVAL OF LOBLOLLY PINE SEEDLINGS ACCORDING TO PRE-STORAGE TREATMENT AND TYPE
OF STORAGE; TREES LIFTED IN APRIL, PLANTED IN MAY, AND RECORDED JUNE 17
CHEMICAL TREATMENTS AS IN TABLE 8

TYPE OF STORAGE	LIVING TREES PER GROUP OF THIRTY										TOTAL NO. LIVING	LIVING (%)
	Vapor treatments					Spray treatments						
	Con-trol	Nam. 0.3	Mix. 0.3	Nam. 0.5	Mix. 0.5	Control (wax)	Nam. 100	Mix. 100	Nam. 600	Mix. 600		
Cold...........	11	9	7	11	13	6	2	5	0	2	66	22.0
Common.......	9	10	10	2	3	3	2	2	0	0	41	13.7
Heeled-in......	1	6	4	4	0	2	0	0	0	0	17	5.7
Total..	21	25	21	17	16	11	4	7	0	2	124	......

Other plants were soaked in water solutions of growth regulators. Inasmuch as past studies at this laboratory had shown roots of pines to be more sensitive to growth regulators than tops, the period of soaking was adjusted accordingly; roots were soaked in the water solutions for 1 hour and tops for 6 hours. The solutions containing 1000 mg./l. of growth regulator were not applied to roots because of the likelihood of injury.

The trees were kept at 32° F. in the cold-storage rooms of the Soil Conservation Service at Beltsville for 54 days. They were planted June 15, in randomized rows of ten trees each, in two replicate blocks to determine effects of the treatments on field performance.

plied to roots only. Similar trends are shown in the plantation mortality data (table 15), particularly for the initial examination 2 weeks after planting.

The combination of poor stock and intense drought resulted in severe mortality by the end of the season, but there remained a small benefit from protective coatings containing no growth regulators. Soaking in water solutions of growth regulators resulted in lower survival than no treatment.

A supplementary test involved dipping a sample of 150 of the stored trees in an emulsion of 50 gm./l. lanolin at the time of planting. The trees so treated were well distributed among some of the original pre-storage treatment groups.

Two weeks after planting, 63% of the trees dipped before planting were dead or dying, in contrast to 92% dead or dying among comparable lots of trees not dipped.

test the effect of pre-storage treatments with both protective coatings and growth regulators applied to tops only, roots only, and whole plants. The trees were placed in cold storage for $5\frac{1}{2}$ months to

TABLE 15

MORTALITY OF SHORTLEAF PINE SEEDLINGS 2 WEEKS AFTER PLANTING AND AT END OF FIRST GROWING SEASON, ACCORDING TO CHEMICALS AND METHODS USED IN PRE-STORAGE TREATMENTS

PART OF PLANT TREATED	WATER-SOLUTION TREATMENTS				SPRAY TREATMENTS					UN-TREATED
					Lanolin (50 gm./l.)			Wax (1:3)‡		
	Nac.*		Mix.†		Mix.			Mix.		
	500 mg./l.	1000 mg./l.	500 mg./l.	1000 mg./l.	None	200 mg./l.	600 mg./l.	None	600 mg./l.	
No. dead 2 weeks after planting										
Tops............	8	7	5	6	1	0	1	0	0	11
Roots and tops.....	8	8	3	8	1	0	2	0	0	6
Roots............	10	......	3	......	8	10	10	8	11	6
Total.........	26	(15)§	11	(14)	10	10	13	8	11	23
No. dead at end of growing season										
Tops.............	20	20	20	20	13	18	20	17	19	17
Roots and tops.....	20	20	20	20	19	20	20	14	20	18
Roots............	20	......	20	......	17	20	20	20	20	20
Total.........	60	(40)	60	(40)	49	58	60	51	59	55

* Naphthaleneacetic acid.
† Mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.
‡ Commercial-wax emulsion diluted with three parts of water.
§ Totals in parentheses are based on groups of forty rather than of sixty trees.

This small test substantiated results of similar tests on other species. It indicated that treatment with inhibiting concentrations of growth regulators before storage is unpromising for pines, but that protective foliage coatings may improve field survival.

3. Pitch pine and tuliptree

PLAN OF EXPERIMENT.—This study, like the preceding one, was designed to provide a rather severe test of the possible protection the treatments might afford against losses during storage.

PROCEDURE.—Several thousand seedlings of 3–0 pitch pine (*Pinus rigida* Mill.) and 2–0 tuliptree (*Liriodendron tulipifera* L.) were lifted from the U.S. Forest Service nursery at Parsons, West Virginia, on April 30, 1943. At the time of lifting, the buds of pitch pine were slightly elongated and many of the tulip-

tree buds were just breaking dormancy. Treatments were accomplished by dipping the seedlings for a few seconds in the various emulsions listed in table 16.

trees was determined by visual examination (table 16).

RESULTS.—An analysis of variance based on the numbers of dead trees

TABLE 16

MORTALITY OF SEEDLINGS OF PITCH PINE AND TULIPTREE DURING 5½ MONTHS' STORAGE AT 32° F., ACCORDING TO PRE-STORAGE TREATMENTS. BASIS: 100 TREES PER GROUP, IN FOUR BUNDLES OF TWENTY-FIVE TREES EACH

COATING MATERIAL	PART OF PLANT TREATED	CONC. OF GROWTH REGULATOR (MG./L.)	PERCENTAGE MORTALITY DURING STORAGE							
			Pitch pine				Tuliptree			
			Growth regulator			Mean	Growth regulator			Mean
			None	Nac.*	Mix.†		None	Nac.	Mix.	
Lanolin (50 gm./l.)	Roots.....	80	12	4	8	8.00	78	80	85	81.00
	Tops......	400	7	10	8	8.33	86	73	77	78.67
	Both......	160	13	18	17	16.00	82	80	77	79.67
	Mean.....	....	10.67	10.67	11.00	10.78	82.00	77.67	79.67	79.78
Controls....	None	0	13	......	......	......	90	......	......	......
Commercial wax (1:3 H₂O)	Roots.....	80	21	30	13	21.33	83	87	94	88.00
	Tops......	400	26	18	20	21.33	84	87	89	86.67
	Both.....	160	51	42	65	52.67	99	97	97	97.67
	Mean.....	....	32.67	30.00	32.67	31.78	88.67	90.33	93.33	90.78
Controls....	None	0	9	......	......	......	90	......	......	......

MINIMUM SIGNIFICANT DIFFERENCES BETWEEN MEANS FOR PITCH PINE:

Criterion	Significant (P-0.05)	Highly significant (P-0.01)
Part of plant..................................	6.2	8.3
Combinations of coating and part of plant.......	8.8	11.7
Combinations of coating, growth regulator, and part of plant.................................	15.2	20.2

* Naphthaleneacetic acid.
† Mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.

After one night of conditioning at 40° F., the trees were transferred to a cold-storage room maintained at 32° F. and 85% relative humidity. On October 20, 1943, the mortality within each group of

showed that only the variation between the two coating materials was significant in the case of tuliptree. Lanolin was superior to commercial wax in preventing losses during storage. Judging from the

equality in survival of control trees and those coated with wax, treatment with lanolin was also superior to no treatment.

A separate analysis of variance of mortality in pitch pine showed high significance of variation due to type of coating, part of plant coated, and the interaction of the two. Approximate minimum significant differences for the significant variables are given in table 17. Differences in mortality attributable to the presence or absence of growth regulators in the coatings were not statistically significant.

Coatings of commercial wax increased mortality of pitch pine, particularly when applied to the whole plant. Lanolin had no significant effect on mortality but showed some tendency to raise it when applied with growth regulators to both tops and roots. In general, however, growth regulators had no appreciable effect on the condition of the stock after $5\frac{1}{2}$ months in cold storage.

Treatment with lanolin emulsion resulted in slightly more mold growth on the roots in comparison with the control trees. The commercial-wax emulsion resulted in moderate to severe molding when applied to roots, probably because of the supply of nitrogen provided by ammonia, which is detectable in the odor of the wax emulsion.

In a test of field survival of pitch pine seedlings treated as described, stored at 32° F., and planted June, 1943, thirteen of eighty controls survived until August, compared with thirty-eight of eighty trees sprayed with lanolin emulsion on tops or tops and roots. The remaining treatments (table 16) were of no benefit.

RÉSUMÉ OF RESULTS OF B: PRE-STORAGE TREATMENTS

1. Attempts to inhibit the production of etiolated shoots on white ash seedlings

kept in unrefrigerated storage by pretreatment with growth regulators were essentially successful.

2. Treatments which resulted in inhibition of shoot growth were vapors of 0.3 and 0.5 mg. of naphthalenemethylacetate and 0.5 mg. of a mixture of growth regulators per cubic foot of storage space, and sprays containing 100 mg./l. of either the acetate or the mixture. Sprays containing 600 mg./l. of the growth regulators were usually lethal.

3. Inhibition of shoot growth was usually accompanied by initiation of new roots above and below the root collar.

4. Trees treated in the fall and those stored outdoors showed less response to growth regulators than trees treated in the spring and those stored in a shed.

5. Among treated and untreated white ash seedlings removed from storage and planted in May during favorable weather, mortality during the first season was uniformly low and not related to pre-storage treatments with growth regulators.

6. Among similar seedlings kept in storage until June, those which received effective inhibition treatments survived significantly better than untreated seedlings during the first few months after planting. After a severe summer drought, however, the differences in survival were no longer significant by October of the first growing season.

7. The pre-storage treatments resulted in higher initial field survival of white ash seedlings which had been kept in cold storage (32° F.) as well as in unrefrigerated storage, even though all trees remained completely dormant during storage at 32° F.

8. Untreated trees kept in cold storage survived better in the field than trees treated with growth regulators but stored without refrigeration.

9. Treatments with growth regulators described in paragraph 2 above usually resulted in lowered field survival of loblolly pine seedlings.

10. Naphthaleneacetic and a mixture of growth regulators in concentrations of 200–1000 mg./l. applied in various ways to seedlings of shortleaf pine usually resulted in lower field survival than in untreated trees.

11. Application of an emulsion containing 50 gm./l. lanolin to dormant shoots of short-

leaf pine, pitch pine, and tuliptree before storage usually resulted in higher survival of the trees during storage and after planting.

C. PRE-PLANTING TREATMENTS

1. *Red, loblolly, and shortleaf pines*

PLAN OF EXPERIMENT.—The previous work on use of growth regulators to delay breaking of dormancy and to inhibit top growth suggested that such treatments might have definite value if applied to forest planting stock just before the trees are set out. It is reasonable to suppose that initial survival might be increased if utilization of stored food by the tops and transpiration of water are decreased through inhibition of shoot growth during the early part of the season while the roots are becoming established. Several studies involving preplanting treatment were therefore conducted concurrently to test this hypothesis.

The plan of the first of these studies included soaking and spray applications of growth regulators to red, loblolly, and shortleaf pine, just before fall and spring planting. Some of the same trees were also tested in the drought machine to help explain the results of parallel tests of survival in the field.

PROCEDURE.—Seedlings of red pine (3–0), loblolly pine (1–0), and shortleaf pine (2–0) were removed from the nursery on October 29, 1942, and sorted into three size classes. Within each class there were thirteen groups of six trees, corresponding to the thirteen treatments shown in table 17.

In making up water solutions of growth regulators, the chemicals were first dissolved in a small quantity of 95% ethyl alcohol. Nevertheless, naphthalene acetamide tended to come out of solution, so it was replaced by naphthalene-acetic acid when these tests were repeated in the spring.

Groups of trees designated for top soaking were immersed to the root collar for 18 hours in the water solutions. Sprays were applied after the trees had been potted individually in sand at 6% moisture content. The top-soaked trees were similarly potted, and all were exposed to 96° F. and humidity of 20% in the drought machine. The three sizes of trees were tested separately in three successive runs of the machine.

In these tests, all plants of one species were removed from the machine at the same time, and each tree was classified in one of seven groups, representing various stages, from no wilting to complete wilting. The seedlings were removed to an unheated greenhouse, where they were transplanted to moist sand to determine which would revive.

Seedlings intended for similar drought-machine tests in the spring were lifted on April 10, 1943, sorted, and treated as before. However, the quantity of lanolin in the sprays was raised to 30 gm./l., for the fall tests had shown that lanolin alone, even in the previous carrier concentration of 5 gm./l., caused a slight increase in drought resistance. After treatment with the solutions and sprays, the trees were heeled-in under a dense pine stand on April 17, for later testing in the drought machine. Since the period of the greatest water stress in spring planting probably comes after new growth has begun to develop, it was planned to conduct the machine tests of the heeled-in seedlings at that stage.

Each of the treatments with growth regulators just described was applied to eighty seedlings of each species to test the effect of the treatments on field survival after fall and spring planting. Immediately after treatment, the trees were

planted in randomized rows of twenty trees in replicate blocks according to an appropriate experimental design.

Fall planting was done November 10 and 11, 1942, and spring planting on April 20 following. The concentration of lanolin in the emulsion sprays was raised

had received the soaking treatments (3.7%) was not significantly different from that of the controls. Any differences in survival between species were purely arbitrary, depending upon the time that the trees of a given species had been removed from the drought machine. Dif-

TABLE 17

NUMBER OF TREES SURVIVING IN GREENHOUSE AFTER TREATMENT WITH
GROWTH REGULATORS AND EXPOSURE TO ARTIFICIAL DROUGHT

		No. TREES SURVIVING							
		Growth regulator						All treatments combined	Controls (not sprayed or soaked)
APPLICATION	SPECIES	Naphthalene acetamide			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion, 5 gm./l.)	Red pine........	3†	2	4	3	6	4	22	2
	Loblolly.........	8	6	3	9	5	7	38	3
	Shortleaf........	4	4	2	3	4	3	20	0
Total......	....	15	12	9	15	15	14	80 24.7%	5 9.3%
Soaking (water solution) 18 hours	Red pine........	0	2	2	2	2	0	8	....
	Loblolly.........	1	1	0	0	0	0	2	....
	Shortleaf........	0	1	0	0	0	1	2	....
Total......	....	1	4	2	2	2	1	12 3.7%	....

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.
† Eighteen trees tested per species and treatment.

from 5 gm. to 30 gm./l. in the spring tests, as previously explained.

RESULTS.—The numbers of trees surviving in the greenhouse 3 months after the fall drought-machine tests are shown in table 17.

Chi-square tests of the ratios of dead and living seedlings showed that survival of the sprayed seedlings (24.7%) exceeded significantly that of controls (9.3%), but that survival of trees which

ferences between the two chemicals and between the three levels were not significant.

These results were substantiated by a summary of top-condition scores representing prescribed symptoms of drought injury to foliage and stems at the end of the drought-machine tests. An analysis of variance of the scores showed that visible injury by drought was least among trees sprayed with lanolin emul-

sion and greatest among those with tops soaked 18 hours in water or solutions of growth regulators (table 18).

Thus, application of lanolin-emulsion spray of low concentration (5 gm./l.), with or without growth regulators, resulted in greater resistance to artificial drought, whereas soaking of the tops for 18 hours in water or in growth-regulator solution probably decreased drought resistance of the seedlings.

One month after other seedlings treated in the fall of 1942 had been planted in the field, top condition of each tree was recorded by the same scoring system used in drought-machine tests. Red pine trees were in uniformly good condition. An analysis of variance of the top-condition scores for loblolly and shortleaf pine (table 19) showed that variation between treatments was highly significant and attributable to the superiority of shortleaf pine seedlings sprayed with lanolin

emulsion over those not treated. Loblolly seedlings were not benefited by the coating.

TABLE 18

SIGNIFICANCE OF DIFFERENCES IN NUMERICAL INDICES OF WILTING OF PINE SEEDLINGS SPRAYED WITH LANOLIN EMULSION OR SOAKED IN WATER SOLUTIONS AND EXPOSED TO ARTIFICIAL DROUGHT. HIGHEST SCORE INDICATES LEAST WILTING. TREATMENTS AS IN TABLE 17

APPLICATION	Av. CONDITION SCORE PER GROUP OF 18 TREES	No. TREES TESTED	SIGNIFICANCE OF DIFFERENCE* WITH	
			Control	Soaked
Sprayed (lanolin emulsion).....	28.3	324	M	H
Soaked (water solution)........	20.9	324	M	......
Control........	24.3	54	......	......

* H, high (1% level); M, medium (5% level).

TABLE 19

NUMERICAL INDICES OF WILTING AND VIGOR OF PINE SEEDLINGS IN PLANTATION 1 MONTH AFTER TREATMENT WITH FOLIAGE COATING AND GROWTH REGULATORS
HIGHEST SCORE INDICATES LEAST WILTING

APPLICATION	SPECIES	MEAN TOP-CONDITION SCORE PER TREE						Mean for all treatments	Controls (not soaked or sprayed)
		Growth regulator							
		Naphthalene acetamide			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion)	Loblolly.........	6.32†	6.15	6.20	6.52	6.50	6.80	6.41	6.42
	Shortleaf........	5.67	4.92	5.40	5.40	5.27	5.40	5.34‡	4.00
	Mean..........	6.01	5.54	5.80	5.97	5.89	6.10	5.88‡	5.21
Soaking (water solution)	Loblolly.........	6.27	6.30	5.00	6.10	6.27	6.42	6.06	6.32
	Shortleaf.........	4.12	3.70	3.67	3.80	4.47	4.10	3.97	3.77
	Mean..........	5.19	5.00	4.33	4.95	5.37	5.26	5.01	5.04

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.
† Basis: 40 trees per group.
‡ Significantly higher than controls (1% level).

By spring, survival of seedlings sprayed with 5 gm./l. lanolin was no longer superior to that of controls or

During the summer, the record drought of 1943 took a very heavy toll of both fall-planted and spring-planted
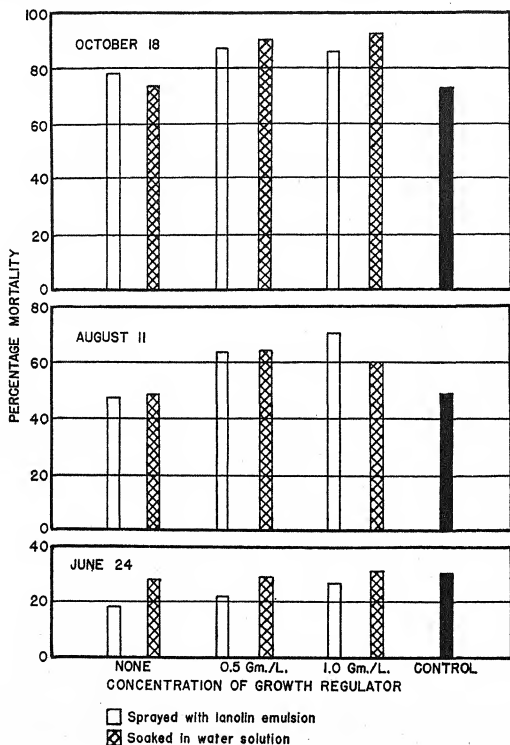
FIG. 10.—Seasonal trend of mortality in seedlings of red, loblolly, and shortleaf pine according to pre-planting treatments with naphthaleneacetic acid or mixture of indolebutyric acid and three naphthalene compounds.

soaked trees. The growth regulators were not beneficial in this respect, and naphthalene acetamide applied by soaking resulted in an increase of 100% mortality.

trees. The progress of mortality for red pine planted in the fall combined with all three species planted in the spring is shown in figure 10. Mortality of loblolly

and shortleaf pines planted in the fall of 1942 was so high by the summer of 1943 that they have been omitted from the diagram in order not to obscure treatment differences.

Until the beginning of the summer, trees sprayed with lanolin and planted in the spring had an evident advantage in survival. However, the presence of a

effect of the severe midsummer drought on unprotected new growth. In other 1943 plantations, not so seriously affected by the drought, the survival advantage was often maintained through the summer.

The complete pattern of effects of different treatments on survival of the three species at different dates of recording is

TABLE 20

SURVIVAL OF RED, SHORTLEAF, AND LOBLOLLY PINES PLANTED IN SPRING AFTER TREATMENT WITH GROWTH REGULATORS, AND RECORDED IN AUGUST, 1943

		No. living trees							
		Growth regulator						Mean for all treatments	Controls (not soaked or sprayed)
APPLICATION	SPECIES	Nac.			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion)	Red pine........	30†	29	34	27	26	28	29.0	32
	Loblolly........	24	1	14	13	21	18	15.2	29
	Shortleaf.......	13	0	0	11	3	0	4.5	8
	Mean........	22.3	10.0	16.0	17.0	16.7	15.3	16.2	23.0
Soaking (water solution)	Red pine........	29	35	16	33	35	36	30.7	31
	Loblolly........	27	0	0	36	6	1	11.7	27
	Shortleaf.......	4	2	0	7	0	0	2.2	2
	Mean........	20.0	12.3	5.3	25.3	13.7	12.3	14.8	20.0

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.
† Basis: 40 trees per species and treatment.

growth regulator in the coating tended to remove this advantage, probably through direct chemical injury. Later in the season the injuriousness of the growth regulators was clearly reflected in an increased rate of mortality of treated trees. Furthermore, the initial advantage of treatment with lanolin alone was lost, as it was in the test of lanolin coating on foliage of loblolly pine in the main storage study. In both these studies mortality of control trees was 70% or more, and removal of the survival advantage of coated trees was probably due to the

too complex for detailed consideration, particularly since the growth-regulator treatments usually resulted in lower survival. However, certain facts are obtainable from table 20. Survival of loblolly and shortleaf pine seedlings was very low and was further decreased by nearly all growth-regulator treatments; the decrease was considerably greater for soaked than for sprayed trees. Survival of red pine was relatively unaffected by the treatments, none of which resulted in consistent increase in survival, as recorded in August, 1943.

Treatment of the dormant seedlings with 500 or 1000 mg./l. of growth regulator before planting caused the same types of shoot inhibition that were described under nursery-bed treatments. The frequency of various degrees of inhibition among the planted trees on June 24 is shown in table 21.

The rather high proportion of control trees which remained dormant or which developed leafless leaders reflected the effects of the transplanting shock and also the rather poor vigor of the loblolly and shortleaf pine seedlings.

All indications are that treatment of pines in the dormant season with 500–

TABLE 21

FIELD PERFORMANCE OF PLANTED RED, LOBLOLLY, AND SHORTLEAF PINES ACCORDING TO LEADER CONDITION IN JUNE, 1943

Treatment	Classification of living trees	Basis (no. living)	Condition of same trees, October 23					
			Living					Dead (%)
			Dormant (%)	Leader present				
				No leaves		Reduced leaf no. (%)	Normal foliage (%)	
				Leader alive (%)	Leader dead (%)			
All growth-regulator treatments combined	Terminal bud dormant	129	0	0	2	0	0	98
	Leader present, no leaves	463	0	1	5	4	0	90
	Leader present, reduced leaf no.	92	0	0	0	18	3	79
	Leader present, normal foliage	27	0	0	0	0	(44)*	(56)
All controls combined	Terminal bud dormant	16	0	0	0	(6)	(19)	(75)
	Leader present, no leaves	30	0	0	(3)	(7)	(13)	(77)
	Leader present, reduced leaf no.	85	0	0	0	4	29	67
	Leader present, normal foliage	415	0	0	0	0	36	64

* Parentheses indicate percentage based on small numbers.

Mortality during the growing season was greatest for trees which initially exhibited the most severe inhibition of leader development (right column in table 21). Practically all trees still dormant in June were dead by October. Two-thirds of the chemically treated loblolly pines which had leafless leaders in June and which survived the summer developed new foliage later in the growing season, but only one-fifth of the red pines did so. On red pines, the leaders which were leafless in June usually died back, even though the tree remained alive.

1000 mg./l. of growth regulators for the purpose of inhibiting shoot development is likely to reduce field survival rather than to increase it. Apparently, the more severe the top inhibition caused by growth regulators, the lower is the survival of pines. The latter finding is subject to a certain bias, since the trees which showed the greatest inhibition may have been the least vigorous ones originally.

The trees intended for the spring series of tests in the drought machine were heeled-in under a fairly dense pine stand

to permit the treatments with growth regulators to take effect on development of new shoots.

By May 31, 1943, 6 weeks after treatment, the typical inhibiting effects of the chemicals on shoot growth were observed. Both concentrations of growth regulator (500 and 1000 mg./l.) applied to dormant seedlings in the spring had practically prevented leafing-out but not shoot elongation of red pine. In loblolly pine, the higher concentration prevented

TABLE 22

MORTALITY (%)* OF RED, SHORTLEAF, AND LOB-
LOLLY PINE SEEDLINGS TREATED WITH
GROWTH REGULATORS AND LEFT FROM APRIL
UNTIL JUNE, 1943, IN SHADED HEELING-IN
BED

SUBSTANCE	CONCENTRATION		
	0	500 mg./l.	1000 mg./l.
Naphthaleneacetic acid.....	9.3	87.0	90.7
Mixture†.................	12.0	65.7	75.9
Mean..................	10.6	76.3	83.3

* Basis: 108 trees per group.
† Same as for table 20.

leafing-out, and the lower concentration reduced the quantity of new foliage per shoot by about two-thirds. Inhibited leaders of loblolly showed the usual tendency to curl, and a few of the leaders were dead. None of the shortleaf pine seedlings had yet leafed out; the trees were therefore left in the heeling-in bed to see what would happen to them as well as to the other pine species.

By June 25, 80% of the chemically treated trees and only 10% of the controls were dead in the heeling-in bed. Naphthaleneacetic acid was somewhat more lethal than the mixture, and the lower concentration was only slightly less

toxic than the higher one (table 22). The lanolin spray without growth regulators resulted in slight but inconsistent increase in survival of the heeled-in trees.

The injurious effects of the growth regulators were distinctly more pronounced in the heeling-in bed than in the plantation (fig. 10). It is likely that the dense shade over the heeling-in bed reduced the vigor of the seedlings and consequently decreased their resistance to inhibiting substances. In view of the fact that treatment with growth regulators to kill undesirable trees, if not to inhibit top growth, should have certain practical applications, it might be observed that dense shade may make the trees much more susceptible to injury by such treatments.

2. Red spruce and tuliptree

PLAN OF EXPERIMENT.—The preceding study of three pine species was paralleled by another involving red spruce (Picea rubens Sarg.) and tuliptree, in order to broaden the applicability of the results. Lanolin and commercial-wax emulsion were used as carriers for the growth regulators, but always in sufficiently high concentrations to serve as protective coatings as well.

An additional feature of the study was the application of coatings to one series of trees immediately after lifting from the nursery, to prevent desiccation during transportation and handling. A brief investigation has already been reported concerning the use of root coatings in lifting and transplanting trees. MILLER, NEILSON, and BANDAMER (9) dipped roots of 2-year-old Norway spruce seedlings in a commercial paraffin-wax emulsion and exposed them to sunshine and wind for periods ranging from 5 to 60 minutes before planting. Survival was several times greater for waxed than for

unwaxed trees exposed in the same manner.

Du Puis (3) found that a solution of 0.1–10% glycerin was effective in protecting the roots of tomato and cabbage plants during storage or shipment. The solution was used in place of water for wetting the packing moss. CHADWICK (1) sprayed roots and tops of shrubs with commercial-wax emulsions before transplanting. Top sprays reduced water loss and usually increased survival. Spraying of roots in addition to tops caused a further decrease in water loss but no further increase in survival.

The present study included application of lanolin and commercial-wax coatings to tops, roots, and whole plants, with and without a mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid. Half the trees were treated just after lifting and the remaining half just before planting or heeling-in. Within each of these two groups, half the seedlings were planted after shipment from the nursery and half were heeled-in 2½ weeks before planting. The four resulting schedules for treatment and planting constituted replications of the experiment under slightly varying conditions. The twelve treatments with chemicals and carriers, applied alike within each replication, are shown in table 23.

PROCEDURE.—Trees for this experiment, provided by the Forest Service Nursery at Parsons, West Virginia, consisted of 2-year-old seedlings of red spruce and tuliptree. The seedlings were lifted April 30, 1943, sorted, and half of them were immediately treated in the packing house by dipping in the emulsions (table 23) for several seconds. Because of rainy weather, the roots had no

chance to dry out between the time of lifting and the time of treating.

Similar pre-planting treatments were applied to the remaining half of the seedlings on May 5 and 6, after they had been packed and transported 200 miles to Beltsville. The two species were planted on separate (though adjacent) areas because a statistical comparison of the performance of a conifer and a hardwood is usually precluded by the unlike ranges of the data on survival and growth. Half the trees were planted May 6 and 7, and the remainder were heeled-in until May 24 before being planted. The plantation provided for randomization and replication of groups of trees which had been treated alike.

RESULTS.—Ten days after the early planting, a record of the top condition of all planted trees revealed that growth regulators applied to tops only or to roots and tops prolonged the dormancy of a portion of the seedlings of tuliptree (table 23).

The effects of the three different treatments with growth regulators on rate of leafing-out of additional trees which had been kept in the laboratory basement are shown in figure 11.

In red spruce, rate of leafing-out was not affected by the treatments with growth regulators, but any coating applied to the roots was soon reflected in browning or death of some of the trees after planting (table 24).

The root coatings evidently impeded the absorption of water, and unless the tops were also coated, the trees were subject to internal drought stress. Foliage coatings tended to result in higher initial survival of spruce in the plantation but not in the shaded heeling-in bed, where transpiration was much lower and where very few uncoated trees died.

When the heeled-in trees were re-
moved for planting on May 24, those of
red spruce showed no visible response to
the growth regulators. As in the planta-

The results of mortality counts of the
planted red spruce trees in June are
given in figure 12. Although random
variation was rather high, as shown by

TABLE 23

DORMANCY OF TULIPTREE SEEDLINGS TREATED WITH PROTECTIVE COATINGS
AND GROWTH REGULATORS, PLANTED MAY 6–7, 1943,
AND OBSERVED 10 DAYS LATER

	PERCENTAGE DORMANT			
TREATMENT	Part of plant treated and conc. of growth regulator			Untreated
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Lanolin emulsion only (50 gm./l.)	18†	10	3	8
Commercial-wax emulsion only (1:3 H₂O)	12	15	10	6
Lanolin plus growth regulator*.	13	28	33	9
Commercial wax plus growth regulator*.	10	22	34	9

* Mixture of equal parts of naphthaleneacetic acid, naphthoxyacetic acid, naphthalene acetamide, and indolebutyric acid.
† Basis: 100 trees per group.
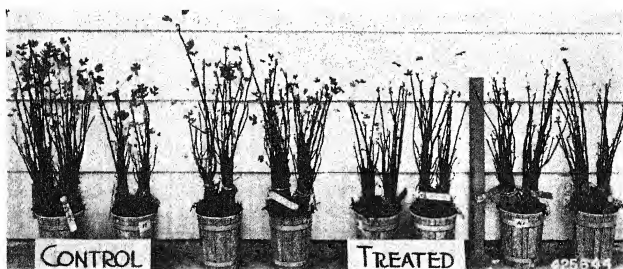
FIG. 11.—Differences in rate of leafing-out of tuliptree seedlings after dipping in emulsion containing mixture of indolebutyric acid and three naphthalene compounds. Reading from left: first pair, untreated; second pair, 80 mg./l. on roots; third pair, 200 mg./l. on roots and tops; fourth pair, 400 mg./l. on tops. Photographed May 15, 1943.

tion, dormancy of tops of tuliptree seed-
lings was more prolonged among those
treated, and a slight increase in growth
of new roots was observed in groups with
roots previously dipped in the chemicals.

the differences among control groups,
mortality to June was distinctly lower
in groups treated on tops only with the
plain foliage coatings.

Mortality of red spruce seedlings re-

corded on August 24, and the results of an analysis of variance of the data, are given in table 25. The drought of 1943 had already taken a fairly heavy toll ing from treatment of only the tops with plain lanolin or wax emulsion, and significantly higher mortality (49.5%) resulting from treatment of whole plants

TABLE 24

MORTALITY AND BROWNING OF RED SPRUCE SEEDLINGS TREATED WITH PROTEC-
TIVE COATINGS AND GROWTH REGULATORS, PLANTED MAY 6–7, 1943, AND
OBSERVED 10 DAYS LATER. CHEMICAL TREATMENTS AS IN TABLE 23

TREATMENT	PERCENTAGE DEAD OR BROWNED			
	Part of plant treated and conc. of growth regulator			Untreated
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Lanolin emulsion only (50 gm./l.)	19*	1	1	22
Commercial-wax emulsion only (1:3 H$_2$O)	20	2	0	5
Lanolin plus growth regulator	30	3	0	5
Commercial wax plus growth regulator	37	0	10	6

* Basis: 100 trees per group.
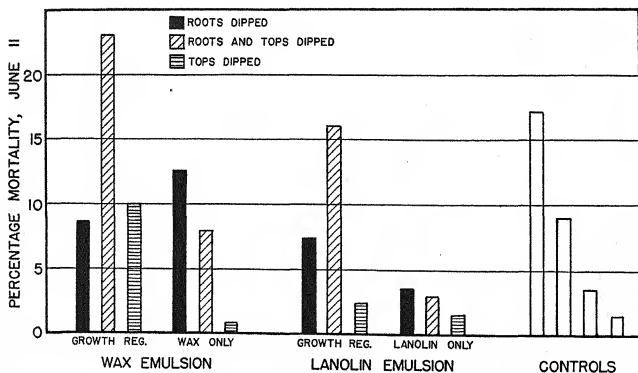
FIG. 12.—Mortality of planted red spruce seedlings in June, 1943, according to pre-planting treatments. Mixture of indolebutyric acid and three naphthalene compounds applied by dipping roots (80 mg./l.), whole plants (200 mg./l.), or tops only (400 mg./l.).

among all groups of trees. In comparison with mortality of untreated trees (20.25%), essential differences were significantly lower mortality (9.5%) resulting with the protective coatings plus growth regulators. As in other studies with conifers in this series, treatment with commercial-wax emulsion resulted in in-

creased mortality—except when applied without growth regulators to the tops only.

In general, trees treated with coatings and growth regulators just before planting survived better than those treated

coatings was largely retained through the summer drought. By October 15, average mortality for red spruce seedlings with protective coatings on the tops only was 11.5% compared with 22.2% for untreated trees.

TABLE 25

MORTALITY OF RED SPRUCE SEEDLINGS TO AUGUST 24, 1943, ACCORDING TO PRE-PLANTING TREATMENTS. DATA FROM TWO MAY PLANTINGS COM-BINED. CHEMICAL TREATMENTS AS IN TABLE 23

	PERCENTAGE MORTALITY				BASIS (TOTAL NO. OF TREES)
TREATMENT GROUP	Part of plant treated and conc. of growth regulator			Untreated	
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)		
Lanolin emulsion only (50 gm./l.)..	8.0	20.5	12.5	33.0	800
Commercial-wax emulsion only (1:3 H_2O).................	31.5	30.0	6.5	15.0	800
Lanolin plus growth regulator.....	17.5	41.5	15.5	11.0	800
Commercial wax plus growth regulator.................	30.0	49.5	34.0	22.0	800
Mean for coatings containing no growth regulator.............	19.75	25.25	9.50*	24.00	1600
Mean for coatings containing growth regulator.................	23.75	45.50†	24.75	16.50	1600
Mean for all coatings............	21.75	35.375†	17.125	20.25	3200

MINIMUM DIFFERENCES ATTAINING SIGNIFICANCE:

Criterion	Significant (P=0.05)	Highly significant (P=0.01)
Part of plant..	.7.6	10.1
Combinations of part of plant and presence of growth regulator........	10.7	14.2
All controls vs. single combinations of part of plant and presence of growth regulator..	9.3	12.3

* Significantly lower than controls (5% level).
† Significantly higher than controls (1% level).

just after lifting, inasmuch as the growth regulators were more harmful on longer than on shorter contact with the trees.

As in most of the 1943 plantations, the survival of groups of red spruce seedlings treated in different ways tended to approach the same level by fall. Nevertheless, the advantage due to foliage

Despite the drought, survival of all groups of tuliptree seedlings was uniformly high (mean 97%), and there was no increase which might have been attributed to the delayed dormancy resulting from treatment of tops with the mixture of growth regulators.

At the end of the growing season, an

analysis of covariance of the length of the old stem and new shoot of 800 tuliptree seedlings showed that the growth regulators and coatings had no significant effect on shoot growth of this species.

Inasmuch as the tuliptree seedlings treated with growth regulators exhibited a slight stimulation of new root growth when they were removed from the heeling-in bed for planting, a sample of trees was dug from the plantation on October 27, 1943, for further study. The sample

of trees. This analysis indicated that differences in dry weight between individual treatment groups were not significant, but that the adjusted mean dry weight for all three treated groups combined was significantly higher (5%~level) than that of the control group. Thus, treatment with growth regulators resulted in a greater dry weight of the treated tuliptree seedlings, although field survival was equally high among treated and untreated trees.

TABLE 26

TOP LENGTH AND DRY WEIGHT OF TULIPTREE SEEDLINGS AT END OF FIRST
SEASON IN PLANTATION, ACCORDING TO PRE-PLANTING TREAT-
MENTS WITH MIXTURE OF GROWTH REGULATORS*

| | PART OF PLANT TREATED AND CONC. OF GROWTH REGULATOR | | | UNTREATED |
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Mean length of old stem (inches)	5.82	7.37	5.90	5.86
Mean dry wt. of seedlings in October (gm.)	7.90	9.55	9.05	6.57
Mean top-root ratio, dry wt. basis	0.267	0.272	0.254	0.274
Mean dry wt. of seedlings after adjustment†...............................	8.37	8.29	9.43	7.00
Basis: no. of trees....................	24	24	24	24

* Same as for table 23.
† Adjusted for initial differences in stem length by analysis of covariance.

consisted of four adjacent rows of twenty-four trees each which had been heeled-in just after treatment and then planted in late May. Oven-dry weights of root, of old stem, and of new stem were determined for each tree, and the length of the old stem was measured (table 26).

Weights of both tops and roots were slightly greater among trees treated with the growth regulators, so that the top-root ratios remained about the same as in untreated treees. The total dry weights were analyzed by the covariance method, using length of old stem as an independent variable to remove variation attributable to initial differences in size

RÉSUMÉ OF RESULTS OF C: PRE-PLANTING
TREATMENTS

1. Inhibiting concentrations (500 and 1000 mg./l.) of naphthaleneacetic acid and a mixture of growth regulators applied by soaking and spraying just before planting did not affect the field survival of red pine seedlings but resulted in lower survival of loblolly and shortleaf pines.

2. These treatments were usually lethal to similar seedlings kept until June in a shaded heeling-in bed.

3. Dipping the tops of the pine seedlings just before planting in an emulsion of lanolin (30 gm./l.) resulted in increased survival during the spring months but failed to protect the trees against a severe drought which occurred in midsummer. Red spruce seedlings with a similar foliage coating containing

50 gm./l. lanolin survived better than controls throughout the first growing season.

4. A commercial-wax emulsion was detrimental to survival when applied to roots of red spruce seedlings.

5. Dipping tops in lanolin or wax emulsions containing 200 or 400 mg./l. of a mixture of indolebutyric acid and three naphthalene compounds delayed slightly the leafing-out of tuliptree but not of red spruce. The delay in leafing did not result in increased survival of tuliptree after transplanting under favorable conditions, but the treated trees were slightly heavier than control trees at the end of the first year.

6. Red spruce seedlings receiving the same treatments with growth regulators as tuliptree usually showed higher mortality in the plantation than controls.

Discussion

A. NURSERY-BED TREATMENT

The prospects for use of growth regulators to prevent excessive top growth of coniferous seedlings in the nursery are not particularly encouraging. In red pine, effective treatments produced trees of distinctly lower dry weight and somewhat lower top-root ratio than controls. The smaller foliage area of treated seedlings did not confer greater drought hardiness during the same summer. It remains to be determined how trees held back in the nursery during one season will perform when planted in the field during a subsequent season.

The primary limitation for use of growth regulators to restrict growth of seedlings in the nursery bed is the danger of killing some of the trees by excessive concentrations of the chemicals, since inhibitory effects are evidently on the borderline of lethality. The curling of leaders of some species of pines (for example, table-mountain pine) after treatment is a limitation in so far as the appearance of the trees is concerned. Another limitation is that growth regulators applied to pines in November and in the spring months did not greatly inhibit elongation of the new leader and did not delay breaking of dormancy. With deciduous trees, however, the prolonged dormancy in treated white ash in the storage studies and in tuliptree in the pre-planting studies could probably be duplicated in the nursery. A slight extension of the spring lifting season would then be possible.

High concentrations of growth regulators afford one means of killing undesirable or excess trees. One or several applications of a wax emulsion or water solution containing 1 gm./l. naphthaleneacetic acid applied during the period of active shoot growth should be lethal to trees in the seedling stage, but these studies give no indication of the concentration required for larger individuals. Studies on herbaceous plants indicate that 2,4-dichlorophenoxyacetic acid is even more effective as a killing agent (5).

B. PRE-STORAGE TREATMENT

Perhaps the most promising application of shoot inhibition to forest-planting practice lies in the prevention of premature shoot growth of hardwood seedlings during long storage periods. The resulting increase in field survival of white ash seedlings may have been due in part to stimulation of root growth by the growth regulators, for even trees kept completely dormant at 32° F. survived better in the field if treated with growth regulators before storage. These findings are in agreement with those obtained by MARTH (8) with rose bushes. The chief limitations shown thus far are the rather wide variation in response of individual trees of the same species and the danger of killing some of the trees. Further experiments on the consistency of the growth response of different lots of seedlings to a given concentration of

growth regulator should precede practical application of the treatments.

Pre-storage treatment of dormant pine seedlings with growth regulators failed to prevent budding-out during storage and appeared to have no advantage.

C. PRE-PLANTING TREATMENT

This survey offers no promise of increased field survival through treatment of pine seedlings with inhibiting concentrations of growth regulators just before planting. Red pine seedlings retain the initial inhibition of leaf growth for the full season, and in this respect they probably characterize the group of northern pines which have only one period of shoot growth per year. Hardwoods, on the other hand, exhibit a delay of only about 2 weeks in shoot growth after treatment, accompanied usually by initiation of additional new roots. One or both of these responses may contribute to increased survival of hardwood seedlings treated with growth regulators before late planting, when premature shoot growth of untreated trees might result in low survival.

Between red pine and similar species which retain the initial inhibition of leaf growth throughout the growing season, and the hardwoods, in which the inhibition is temporary, there is a group of pine species (for example, loblolly pine) which resume normal growth several months after the initial inhibition is induced. The expectation of improved survival of such seedlings, which might result from a temporary reduction in transpiration until the roots became established, was not realized in these studies. In general, treatment of pine seedlings with inhibiting concentrations seemed to reduce the vigor of the seedlings and often killed some of them because of irregularity in sensitivity of individual trees. Although risk of some mortality may be permissible in treating pine seedlings which must be held for an extra year in the nursery bed, this risk could not be afforded in treating trees which are about to be planted.

Dipping the foliage of seedlings of conifers in emulsions of lanolin or commercial wax before planting seems to afford measurable protection from mortality during the spring months, when the trees are becoming adjusted to field conditions. This protection diminishes during the summer months. In the studies reported here the coatings were employed incidentally as carriers of growth regulators; optimum concentrations for protection of foliage were not specifically determined. Application of coatings to the roots was generally of no benefit, and the commercial-wax emulsion increased mortality of coniferous seedlings when applied to the roots. COMAR and BARR (2) found that injury to foliage of *Helianthus annuus* sprayed with a wax emulsion was attributable to ammonium linoleate in the spray.

Summary

1. A series of seven experiments, conducted between October, 1942, and October, 1943, involving about 25,000 individual trees and several hundred feet of nursery bed, was undertaken to survey several potential applications to forest-planting practice of the control of top development of seedlings by means of growth regulators. Seedlings of red, loblolly, shortleaf, pitch, and table-mountain pines, red spruce, tuliptree, and white ash were treated in the nursery bed, before storage or before planting. Napthaleneacetic acid, naphthalenemethylacetate, napthalene acetamide, and mixtures of naphthalene

compounds with or without indolebutyric acid were applied in various ways. Methods included dipping, soaking, or spraying with water solutions and carrier emulsions of lanolin or commercial wax, and exposure to vapors of the compounds.

2. Nursery-bed treatments were applied for purposes of prolonging dormancy and restricting the amount of shoot growth of pine seedlings. The normal surge of leader growth of pine seedlings was not delayed or substantially lessened by concentrations of 200 or 600 mg./l., sprayed on the expanding buds and old foliage. Needle development, once started, could be completely arrested shortly after treatment and "frozen" for the remainder of the season on red and table-mountain pines. Undesirable curving of the new leaders resulted after treatment of table-mountain pine but not of red pine.

3. At the end of the growing season, red pine seedlings which had been sprayed in May with 600 mg./l. naphthalene acetamide had somewhat lower top-root ratio and much lower average dry weight than untreated seedlings. Inhibiting treatments were close to the lethal concentration, for they occasionally killed some of the trees. More than one application during the period of shoot growth of a spray containing 600 mg./l. naphthaleneacetic acid or similar growth regulators resulted in death of most of the seedlings.

4. Red pine seedlings having only one-half to one-third the normal quantity of new foliage, as a result of inhibition by growth regulators, were no more resistant to artificial drought than untreated seedlings. Top pruning of loblolly pine during early spring resulted in significant decrease in resistance to artificial drought and to transplanting during

the same summer and therefore did not appear to be a desirable alternative to growth regulators for checking top growth in nursery beds.

5. Vapors and sprays of naphthalene-methylacetate and a mixture of growth regulators were effective in preventing or restricting the development of etiolated shoots of white ash seedlings held until June in unrefrigerated storage. These treatments also stimulated initiation of new roots, but a high proportion of the new roots appeared on the stem. Inhibition of shoots and stimulation of roots were much less distinct in treated trees heeled-in outdoors. Pre-storage treatments with growth regulators did not affect the survival of white ash seedlings planted in May when controls were still largely dormant, but they resulted in higher initial survival and greater root growth of trees planted in June.

6. For 2-year-old seedlings of white ash, a successful pre-storage treatment was naphthalenemethylacetate applied as vapor (0.3 or 0.5 mg./cu. ft. of space for 18 hours at 70° F.) or as a spray (100 mg./l. in dilute commercial-wax emulsion). Similar pre-storage treatments did not improve survival of loblolly or short-leaf pine seedlings.

7. Growth regulators were also applied just before planting in an attempt to improve initial survival by holding back top growth and transpiration while the roots become established. Pre-planting treatments were applied to tops of seedlings of red, shortleaf, and loblolly pines, and to roots, tops, and whole plants of red spruce and tuliptree. Various treatments with 80-1000 mg./l. naphthaleneacetic acid and other compounds failed to improve the field survival of conifers, and usually lowered it.

8. Seedlings of tuliptree dipped in emulsions containing 200 or 400 mg./l.

of a mixture of growth regulators just before planting in the spring leafed out more slowly than untreated trees. However, survival was so uniformly high for all treatment groups (planted in May) that no advantage resulted from the prolonged dormancy. Tuliptree seedlings treated with growth regulators showed slight stimulation of root growth in the heeling-in bed and excelled controls in total dry weight by the end of the first season in the plantation.

9. Application of emulsions of lanolin (30–50 gm./l.) or commercial wax to foliage of conifers immediately before planting resulted in higher survival during the spring months. After a long and severe summer drought (1943), the survival of coated seedlings exceeded that of controls in some plantations but not in others.

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ALKALOID CONTENT OF ECUADORAN AND OTHER AMERICAN CINCHONA BARKS

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Introduction

Prior to 1942 and the war in the Pacific, more than 90% of the world supply of quinine and related antimalarial alkaloids came from the Dutch East Indies. There on plantations was grown a strain of *Cinchona officinalis* L., sometimes described as Cinchona Ledgeriana Moens. As a result of controlled breeding and selection, clones of much higher alkaloid-producing capacity were developed than have as yet been found in the remaining wild stands now being exploited in South America.

Without access to the East Indies, the United States and its allies found themselves dependent on synthetic antimalarials and on supplies of American bark from the Andean forests of South America. A few plantations had been started in Central and South America, but the majority were too young for harvest and none extensive enough to supply the wartime demands. Exploration parties were therefore sent by the former Board of Economic Warfare, an agency of the United States government,[3] into the Andean countries to search out wild stands of cinchona. Thousands of tons of South American bark have been located and harvested, much of it from areas previously unexplored and unexploited.

The distribution of cinchona in western South America has been found to be very widespread, occurring as a minor constituent of the rain-forests from Bolivia north to Colombia and Venezuela. Where rain-forests occur, cinchona species are found on both east and west slopes of the Andes at elevations of 150 feet to more than 10,000 feet. It will be for the systematic botanist to decide how many distinct species occur in the Andean region and their exact distribution. Much natural hybridization may have taken place and many local varieties or types now exist.

One of the most striking characteristics of cinchona bark chemically is its diverse composition. The layman may think of cinchona as the tree that yields quinine. Actually, quinine is often a very minor constituent or is entirely absent in the bark of many true cinchona species. Three alkaloids of antimalarial value commonly occur in American cinchona barks—cinchonine, cinchonidine, and quinine. In addition, quinidine, an isomer of quinine used in treating auricular fibrillation, is present in small amounts in some barks. Cultivated or improved varieties may yield as much as 13% or 14% of crystallizable alkaloids, with more than 90% in the form of quinine. On the other hand, many wild cinchonas contain mere traces of crystallizable alkaloids and no quinine. The term "total crystallizable alkaloids" is used in this paper as the sum of the per-

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centages of anhydrous quinine, quinidine, cinchonine, and cinchonidine.

Cinchonine is very prevalent in native Ecuadoran barks, together with its optical isomer cinchonidine. Some barks contain quinine as well as cinchonidine and cinchonine, while a few contain traces of quinidine. Some barks contain all four alkaloids, others three, while still others have only two. In many cases nothing but cinchonine is present, while in barks of a few true cinchonas none of the four common alkaloids are detectable.

This paper presents data collected in Ecuador by the Foreign Economic Administration and the Office of Foreign Agricultural Relations of the United States Department of Agriculture, together with data from other Latin American countries, which illustrate the comparative variability in composition and alkaloidal content of the species and varieties encountered. The data may be of value in evaluating these species as material for breeding and selection programs in cinchona culture. In addition, data will be presented showing the relative proportions of the several various alkaloids present, which may serve as criteria to support or verify species and variety delimitation.

Investigation

I. DISTRIBUTION AND COMPOSITION OF ECUADORAN CINCHONA BARKS

In the course of the operation of the Foreign Economic Administration Cinchona Analytical Laboratory in Quito, more than 1000 bark samples have been analyzed. This total is made up of barks of three principal species—*Cinchona pubescens* Vahl., *C. officinalis* L., and *C. pitayensis* Wedd., together with small numbers of unidentified species as well as species of no commercial value. The

approximate compositions of the most common Ecuadoran commercial types within each species are shown in table 1, with analyses of a few other American barks for comparison.

CINCHONA PUBESCENS.—This is the most common and widespread species of cinchona in Ecuador. A number of types or varieties may be recognized. The first three of these (table 1) contain only cinchonine, although occasionally samples, perhaps hybrids, are taken which show traces or small amounts of quinine and cinchonidine. The Bofuda type is widespread on the west slope of the Andes from southern Colombia to central Ecuador, at elevations of 5000–8000 feet, but has been little exploited because of its low alkaloid content and lack of quinine. The Rosada type occurs in southern Ecuador on both slopes of the Andes at 4000–8000 feet. Much bark of this type has been harvested for use in totaquina manufacture, in spite of its relatively low alkaloid content and lack of quinine. The Serrana occurs at elevations of 8500–10,000 feet on the west slope in central Ecuador. This regularly contains more than 3% cinchonine and has been extensively harvested in spite of its lack of quinine.

The Roja type of *C. pubescens* is the only commercial type of this species in Ecuador that regularly contains substantial amounts of quinine. It occurs on the western slopes of the Andes, from the Colombian border to southern Ecuador, and in the Colonche Hills—an isolated range between the Guayas Estuary and the Pacific Ocean. It grows at elevations of 2500–4000 feet and has been cultivated for years in small plantations in the Andean foothills of central Ecuador. About 1860, the Englishman SPRUCE (4) took seeds and seedlings of this type to India, where it was planted under the name of *Cinchona succirubra*.

Extensive plantings were made in Guatemala in the late nineteenth century. One such planting was at Finca El Porvenir, where much natural seeding and regeneration took place. Extensive wild stands developed. These have been harvested and studied during the present war by the Foreign Economic Administration. A comparison of the alkaloidal content of the Guatemalan Succirubra

8500–10,500 feet on both eastern and western slopes of the Andes. Again, as with *C. pubescens*, this species is primarily a cinchonine-producer but with quinine as the second alkaloid and with cinchonidine present in lesser amounts. Very small amounts of quinidine are usually found. This bark is being extensively harvested, as it has an average of at least 5% total crystallizable alkaloids and is

TABLE 1

APPROXIMATE COMPOSITION (% DRY WEIGHT) OF ECUADORAN
AND OTHER AMERICAN COMMMERCIAL CINCHONA BARKS

SPECIES AND TYPE	SOURCE	No. SAMPLES	INDIVIDUAL ALKALOIDS PRESENT				TOTAL CRYSTALLIZABLE ALKALOIDS
			Cinchonine	Quinine	Cinchonidine	Quinidine	
C. pubescens Bofuda	Ecuador	36	2.12	....	....	....	2.12
C. pubescens Rosada.......	Ecuador	165	2.72	....	....	....	2.72
C. pubescens Serrana.......	Ecuador	60	3.30	....	....	....	3.30
C. pubescens Roja.........	Ecuador	31	1.96	1.48	1.57	....	5.01
C. pubescens Succirubra....	Guatemala	37	2.47	1.71	2.16	....	6.34*
C. pitayensis..............	Ecuador	92	2.63	1.68	0.55	0.19	5.05
C. officinalis Hoja de Lucma	Ecuador	78	1.16	0.41	1.12	....	2.69
C. officinalis Baños........	Ecuador	42	1.36	0.91	1.69	Trace	3.96
C. officinalis Uritusinga.....	Ecuador	10	0.31	1.42	0.67	....	2.40
C. officinalis Costrona Fina..	Ecuador	32	0.12	1.58	1.06	....	2.76
C. officinalis Calisaya.......	Bolivia	52	0.55	3.35	0.83	0.07	4.80†
C. officinalis Ledgeriana.....	Peru	80	0.69	6.90	1.43	0.29	9.31‡

* Courtesy Foreign Economic Administration Analytic Laboratory, El Porvenir, Guatemala.
† Courtesy U.S. Food and Drug Administration and Foreign Economic Administration.
‡ From Report on Punizas Cinchona Planting (in press).

with the Ecuadoran bark of the same variety is shown in table 1. This variety appears to be primarily a cinchonine-producer, as are other types of *C. pubescens*, but with the optical isomer cinchonidine a close second and with quinine usually the minor alkaloid in both Ecuadoran and Guatemalan bark. Samples from Guatemala show slightly higher percentages of each alkaloid than do the limited number of Ecuadoran samples.

CINCHONA PITAYENSIS.—This species occurs extensively in southern Colombia and northern Ecuador, at elevations of

the best source of quinine of any bark at present available in Ecuador.

In leaf characteristics, flower, fruit, and seed appearance, *C. pitayensis* is similar to the Serrana, which is reported as a *C. pubescens* type in central and southern Ecuador in the same elevation zone.

CINCHONA OFFICINALIS.—This species is widespread in southern Ecuador on both eastern and western slopes of the Andes and in central Ecuador on the eastern or Amazon side. A number of distinct types occur at elevations of 4000–

9000 feet. Some, identified as *C. officinalis*, contain practically no alkaloids, while others contain more than 4%. Only the four types extensively harvested will be discussed.

Of the types of *C. officinalis* occurring in southern Ecuador, one (known as Hoja de Lucma) contains on an average equal parts of the optical isomers cinchonine and cinchonidine, with quinine always a minor alkaloid. While this bark seldom contains more than 3% total crystallizable alkaloids, it has been extensively harvested on the eastern slopes of the Andes and to a lesser extent on western slopes and Andean spurs extending toward the Pacific. Recent collections[4] suggest that this may be *C. lucumaefolia* Pavon, by some considered a distinct species, rather than a type of *C. officinalis*. A bark of very similar composition occurs in the Amazon rain-forests of central Ecuador below Baños, where a road penetrates the eastern cordillera and where considerable recent exploitation has taken place. The latter bark contains almost 1% quinine on the average, but usually both cinchonine and cinchonidine exceed the percentage of quinine present.

Among the other *C. officinalis* types of varieties of southern Ecuador, two quinine-producing barks occur. Near Loja a bark known locally as Uritusinga has been harvested since about the year 1640 and marketed as Loja bark, or crown bark, following its use to effect one of the first malarial cures on record in the neighboring town of Malacatos, about 1600 (5). The stands of the Uritusinga variety have been largely cut out, but they originally formed part of the rain-forest at or near the continental divide south of Loja, at 7000–9000 feet. Present samples of this

bark are characterized by a rather low alkaloid content, but with quinine as the major alkaloid present and with smaller amounts of cinchonidine and practically no cinchonine (table 1).

In the high Amazon rain-forest east of Cuenca, a bark of similar composition is now being harvested extensively at elevations of 7000–8500 feet. This type, known as Costrona Fina, is quite distinct from the Uritusinga of Loja in leaf, flower, and fruit characteristics, and in its growth. It has almost identical bark composition, however, with quinine as a major alkaloid and small amounts of cinchonidine, while cinchonine is almost entirely absent.

The resemblance of these two Ecuadoran quinine barks to the Calisaya type of *C. officinalis* of Bolivia and the Ledgeriana strain cultivated in Peru is shown in table 1. Costrona Fina and Uritusinga contain little more than half the alkaloid content of the Bolivian samples, but the proportion of the alkaloids present is similar. Also, the cultivated Ledgeriana strain of *C. officinalis* resembles closely the composition of the Calisaya strain, from which it probably arose; but selection and breeding have raised average percentage of quinine and other alkaloids almost to twice that of the parent type, according to the limited number of samples available for comparison.

II. VARIATION IN BARK COMPOSITION

The Ecuadoran commercial samples, the average values of which have been presented in the preceding section, were either group samples taken by field parties to assay cinchona stands encountered in exploration or were aliquot samples taken from lots of bark at time of purchase. Considerable variation—both in total crystallizable alkaloid content and in individual alkaloids—entered the fig-

ures listed as approximate composition in table 1. In order to evaluate the species and varieties or types for selection or breeding work, or for use in experimental plantings as seedling populations, the amount and type of variation present must be considered.

VARIATION IN COMMERCIAL SAMPLES. —Most Ecuadoran barks have been harvested either as sources of quinine or for use in the preparation of totaquina. This product is a mixture of natural cinchona alkaloids. It contains not less than 7% and not more than 12% anhydrous quinine, and a total of not less than 70% and not more than 80% of the anhydrous crystallizable cinchona alkaloids—quinine, quinidine, cinchonine, and cinchonidine (7). Accordingly, in considering the variability of commercial barks, data both as to total crystallizable alkaloidal content and as to quinine will be presented.

Probably the commonest and most widespread of the Ecuadoran barks are the cinchonine. These rarely contain appreciable quantities of other alkaloids, although occasionally traces of quinine may be found where samples were taken from regions bordering on stands of quinine-producing cinchona types. The commonest cinchonine barks are the Bofuda, Rosada, and Serrana types. Block graphs showing the variability of these common barks in total crystallizable alkaloid content are shown in figure 1. Actually, these values represent only cinchonine.

The Bofuda type is the poorest of the cinchonine barks and shows a variation of from 1% to 4.5% total crystallizable alkaloids. However, 86% of the samples contained 1.5-3.0%. In all, only thirty-six samples of this type of bark were available for study. The Rosada type contains slightly more alkaloids than Bofuda, and 84% of the 164 samples

analyzed fell between 2.0% and 3.5%. Both are undoubtedly variant types of *C. pubescens*.

The Serrana type shows (fig. 1) a range from 1% to 5% total crystallizable alkaloids but contains more samples in the higher classes than did either of the other cinchonine barks. Of the sixty samples studied, 77% contained more than 3% total crystallizable alkaloids or cinchonine. This bark has been considered a type of *C. pubescens* and is so listed here because of similarity of composition. In reality, the tree in leaf, fruit, and flower characteristics is much closer to *C. pitayensis*, which occurs in northern Ecuador in the same elevation zone.

On the basis of the commercial samples, it would appear that the Serrana type offers a considerably better possibility for selection of high cinchonine-producers than either Bofuda or Rosada. Alkaloids other than cinchonine are rarely encountered. Cinchonine could be readily and cheaply extracted in pure form by a simple process if there were a demand for it.

The range in alkaloid composition of the four most widely harvested Ecuadoran barks containing quinine is shown in figure 2, which illustrates the variability in alkaloid content. For each bark, the values for quinine are plotted above the corresponding total crystallizable alkaloid values, so that an idea may be gained as to the value of the bark both as source of quinine and as raw material for totaquina manufacture.

The Roja type of *C. pubescens* and the samples of *C. pitayensis* show in figure 2 about the same range of variability. Both populations had some samples of nearly 7% total crystallizable alkaloids. The block graph of the *C. pitayensis* is quite regular and indicates an almost normal distribution of the population of ninety-

two around the central class of 4.5–5.0% total crystallizable alkaloids. The *C. pitayensis* samples contained a little more quinine than those of the Roja type of used in selection work of plant materials suitable for totaquina production.

The two types of *C. officinalis* illustrate a marked difference in alkaloid-
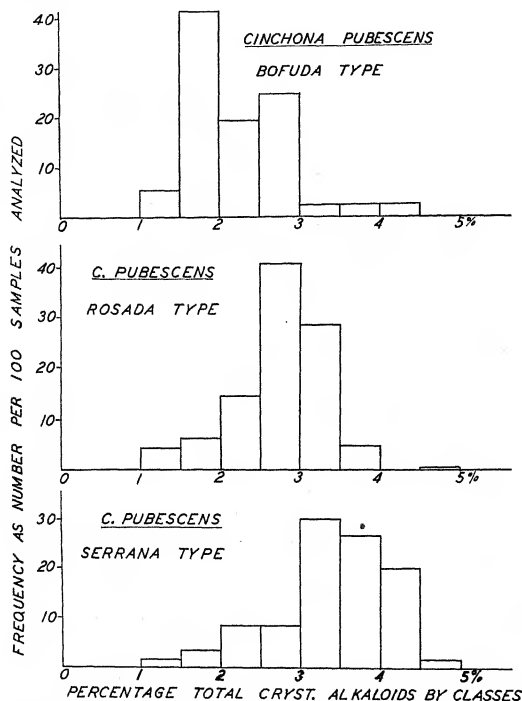
Fig. 1.—Variation in total crystallizable alkaloid content of Ecuadoran cinchonine barks

C. pubescens. Only 9% of the thirty-one Roja samples contained more than 2% quinine, while more than 33% of the *C. pitayensis* samples exceeded this value. Neither shows promise as source material for quinine extraction but could well be forming characteristics. Both the Hoja de Lucma and the Costrona Fina are low alkaloid-producers and show approximately the same range of variability in total crystallizable alkaloid content. However, none of the seventy-eight Hoja
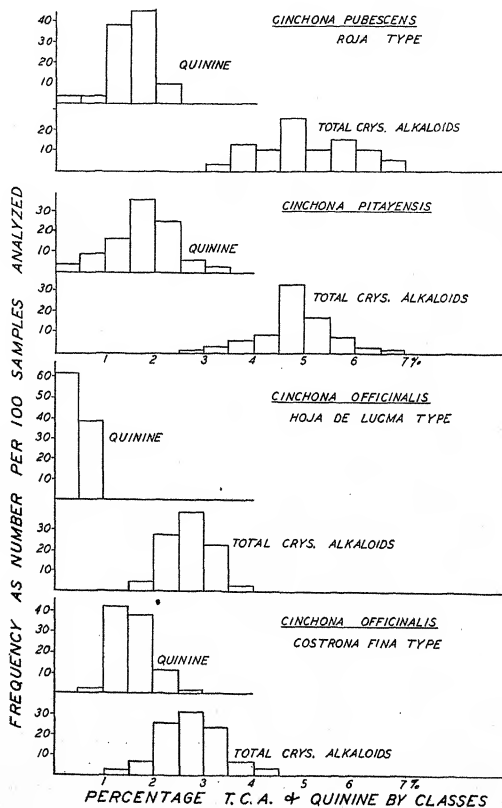
Fig. 2.—Variation in total crystallizable alkaloid and quinine content of Ecuadoran commercial barks

de Lucma samples contained as much as 1% quinine, while nearly all the forty-two Costrona Fina samples exceeded this figure. In this latter type, 82% of the population contained 1–2% quinine, while in 13% of the cases more than 2% quinine was found. As a commercial quinine source such bark is not promising, but it does offer nearly as good a source of quinine as either *C. pitayensis* or the Roja type of *C. pubescens*.

VARIATION IN INDIVIDUAL TREES.—While the assay of commercial group samples may provide information as to the usual composition of a bark, and may indicate the range of variability to be expected, it can hardly serve as an accurate guide in planning selection work to build up clones of high alkaloid-producing capacity. Accordingly, data are presented showing the composition of bark from individual trees of the two most promising types encountered in the preceding section. Also, in figure 3 are data from a group of cultivated trees of the Ledgeriana type of *C. officinalis*.

A group of fifty-seven wild trees of *C. pitayensis* were sampled in a single stand in Ecuador to determine in a preliminary way the possibility or feasibility of clone selection in this species. These were growing in a group of groves mixed with other forest trees along a single ridge at about 9500 feet. As the trees were felled in commercial harvest operations, a bark sample at 1-m. height around the circumference of each tree was taken and softwood cuttings cut from the tree top and placed in nurseries adjacent to the harvest area. Data from the analyses of these bark samples are shown in figure 3. The population sampled was apparently made up of two types of tree, as may be seen from the figure. Nine of the trees contained less than 2% total crystallizable alkaloids, mostly cinchonine, while

the remaining trees showed a range from 2% to 8%, much the same as observed in the commercial series of samples of this species (fig. 2). No trees of unusually high quinine content were encountered.

Through the courtesy of the Foreign Economic Administration, data have been made available to secure an assay on the possibilities of clone selection in the Succirubra type of *C. pubescens*. This type of cinchona was widely planted in Guatemala in the late years of the nineteenth century (3). At Finca El Porvenir in northwestern Guatemala it escaped from cultivation, and large wild stands developed in the mountains adjacent to the original plantings. In connection with the propagation program of the Foreign Economic Administration at El Porvenir, a group of 314 wild trees was sampled and analyzed individually in a clone-selection program carried out under the direction of Mr. WILLIAM PENNOCK, Horticulturist, and Mr. WALTER PLOCHARSKI, Chemist. Permission to use their unpublished data for a comparison with other material in this report is gratefully acknowledged.

This population of Succirubra trees varied widely in total crystallizable alkaloid content (fig. 3). Total values for individual trees varied from 1.7% to more than 11.5%. The graph indicates a very nearly normal distribution pattern. Nearly 4% of the population was made up of trees with over 9.5% total crystallizable alkaloids. Quinine analyses were made on bark of 111 of the best trees, and the variation in quinine content (fig. 3) indicates that this type of *C. pubescens* offers little possibility as a commercial quinine source. In no case did the maximum quinine values approach the high total values encountered. It is evident that Succirubra does offer

excellent possibilities for the establishment of clones for the production of high alkaloid bark suitable for totaquina manufacture.

in total crystallizable alkaloids and quinine content is presented in figure 3. A range from 0.3% to 13.0% total crystallizable alkaloids was encountered. Only
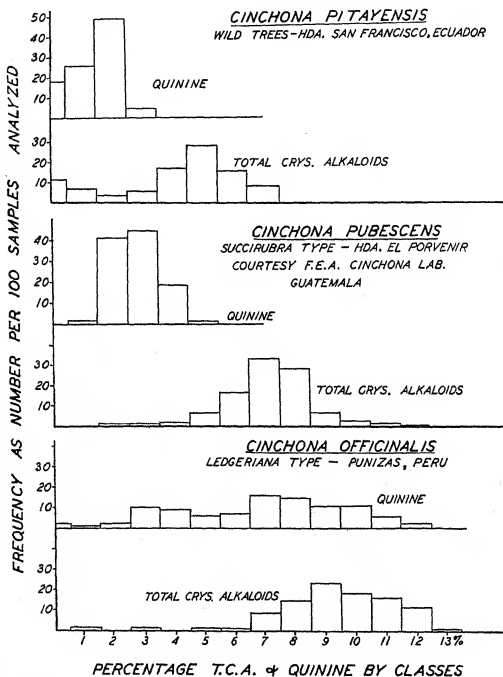
FIG. 3.—Variation in total crystallizable alkaloid and quinine content of bark of individual trees

In 1942, groups of eighty Ledgeriana trees were sampled individually on a small plantation in Peru by MARTIN and CRANDALL (1). The description of the planting and the results of bark analyses form the subject of a separate report (2). For comparison, the reported variability

a small part of this variability could be accounted for as related to age of tree. More than 94% of the population yielded samples of more than 6.5%, while 29% of the trees gave values in excess of 10.5%.

A large proportion of the alkaloids present in the bark of these Ledgeriana

trees was present as quinine. Figure 3 shows that the quinine values in many cases approach the total maximum values. It is reported (2) that in two-thirds of the population, quinine accounted for more than 70% of the crystallizable alkaloids present, while in nearly one-third of the trees more than 90% was found in the form of quinine. Obviously, such a population offers a far better possibility for the selection of quinine-producing clones than does either Succirubra or *C. pitayensis*.

III. Alkaloid ratio diagrams as aid to species and variety delimitation

In the preceding section, data have been presented showing the range of variability in composition of the principal Ecuadoran cinchona barks, together with limited data from other Latin American countries for comparison. In studying these data it was observed in some cases that, where one alkaloid was present in larger amounts than usual, another might be correspondingly low, with the result that the total crystallizable alkaloid value of the sample did not differ materially from that usually typical of the species or type of bark. In other cases within a group of like samples, some of rather low or unusually high total content were observed. In such samples the relative proportions of the constituent alkaloids often remained about the same as that usual for the species or type. This suggested the advisability of considering alkaloid accumulation and formation characteristics apart from the actual percentages and on a relative basis.

Accordingly, the data have been recalculated, expressing the three individual alkaloids—quinine, cinchonine, and cinchonidine—as percentages of the values for total crystallizable alkaloids. In cases

where quinidine was present in very small amounts, the alkaloid percentages were calculated from the sum of the three principal alkaloids. The data so obtained have been plotted on triangular co-ordinate charts. In this way it is possible to plot the percentages of quinine, cinchonine, and cinchonidine at the same time. The position of the resultant dot indicates the composition of the sample with respect to the relative proportions of its three constituent alkaloids. Thus, in figure 4, if a dot were to fall at any apex of the triangle it would be composed of but a single alkaloid. On the other hand, if a dot were to fall at the exact center of the triangle, it would represent a sample composed of equal parts of quinine, cinchonine, and cinchonidine. Points falling on the sides of the triangle would be made of only two alkaloids. In figure 4 are plotted the points representing the commercial Ecuadoran samples of *C. pitayensis* and of the Hoja de Lucma and Costrona Fina types of *C. officinalis*.

The ratios or relative proportions of the three principal alkaloids of ninety-two trees of *C. pitayensis* are shown by the position of the ninety-two solid dots in figure 4. It will be noted that nearly all the dots fall in the same zone, the one in which the percentages of both quinine and cinchonine exceed that of cinchonidine. A measure of the variability in alkaloid-forming characteristics of this species is shown by the scatter of the points on the chart. A greater tendency to form cinchonine than quinine may be noted. No sample contained more than 60% of its alkaloids as quinine, while nearly one-third of the samples showed more than 60% of the alkaloids present in the form of cinchonine. No relation of actual total crystallizable alkaloid content of the bark to the position of the dots was noted.

The two types of *C. officinalis* present a contrasting picture, as far as the alkaloid proportions are concerned. In figure 2 it was shown that the Costrona Fina type had essentially the same total crystallizable alkaloid content as the Hoja de Lucma type. The difference in the alkaloids present in the two barks is clearly shown in figure 4. That the Costrona Fina type is primarily a quinine-cinchonidine producer may be seen by the series of triangles along the quinine-cinchonine axis. Relatively few of the samples contained any substantial pro-portion of cinchonine, and 84% showed more quinine than cinchonidine. Again, there was no detectable relation between total crystallizable alkaloids and the po-sition of the small diagrams on the chart.

The Hoja de Lucma bark contained an average of about equal parts of the two isomers, cinchonine and cinchoni-dine, according to the approximate anal-ysis shown in table 1. However, it is evi-dent from the data of figure 4 that sam-ples of this type of *C. officinalis* range from high cinchonine and low cinchoni-dine at the left of the chart to low cincho-
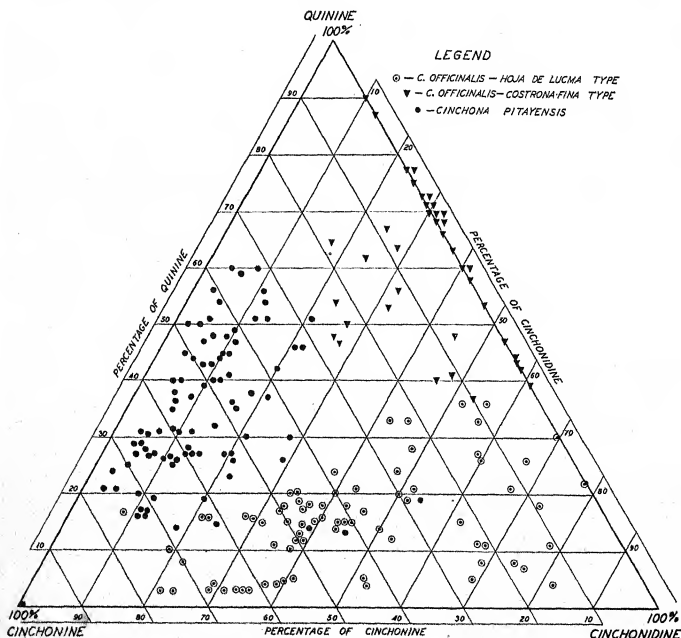
FIG. 4.—Alkaloid ratio diagram for Ecuadoran *C. pitayensis* and two types of *C. officinalis*

nine and high cinchonidine at the right. Very few samples showed any substantial proportion of quinine, and in 82% of the population both cinchonine and cinchonidine exceeded quinine. Whether the Hoja de Lucma is a true *C. officinalis* or a variant type previously described as *C. lucumaefolia* (5) is for taxonomists to decide. It does appear that in alkaloid-forming characteristics the two types are distinct.

Another type of alkaloid ratio pattern is presented in figure 5. Here a comparison is made between the Guatemalan Succirubra type and the Ecuadoran Roja type of *C. pubescens*. The Roja points were the same set of commercial samples whose average alkaloid content and variability in composition are shown in table 1 and figure 2.

The Succirubra samples were from a series of twenty-seven individual trees sampled by PLOCHARSKI and STEVENSON in a study of the alkaloid content of Succirubra trees at El Porvenir, Guatemala. Permission from the Foreign Economic Administration to use these unpublished data is gratefully acknowledged.
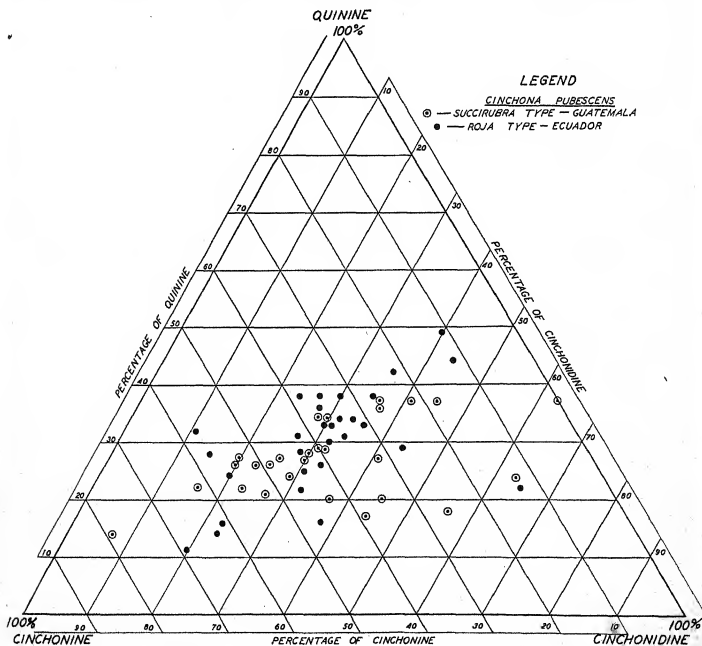
FIG. 5.—Comparison of Ecuadoran Roja with Guatemalan Succirubra type of *C. pubescens*

Both series of points in figure 5 exhibit considerable scatter but tend to group about a general value of 40% cinchonine, 30% cinchonidine, and 30% quinine. The scatter patterns of both types of *C. pubescens* are so similar that they seem to corroborate the record that Succirubra was taken from Ecuador in 1860 (4) to India and returned to the Western Hemisphere in an importation from Ceylon to Guatemala in 1883 (3). No relation between the position of the dots of either series with total crystallizable alkaloid content was found, and the variation indicated by the scatter

seems to be probably due to the genetic variability within this type or variety.

A comparison of the Hoja de Lucma type of *C. officinalis* with another Ecuadoran type of similar bark composition occurring in the Baños area of Ecuador is made in figure 6. This latter has been considered a type of *C. officinalis* and shows a very similar scatter pattern when compared with Hoja de Lucma in the diagram. The Baños type tends to have a slightly larger proportion of quinine. Although the Baños type contained an average of 3.96% as compared with only 2.67% of total crystallizable alkaloids in
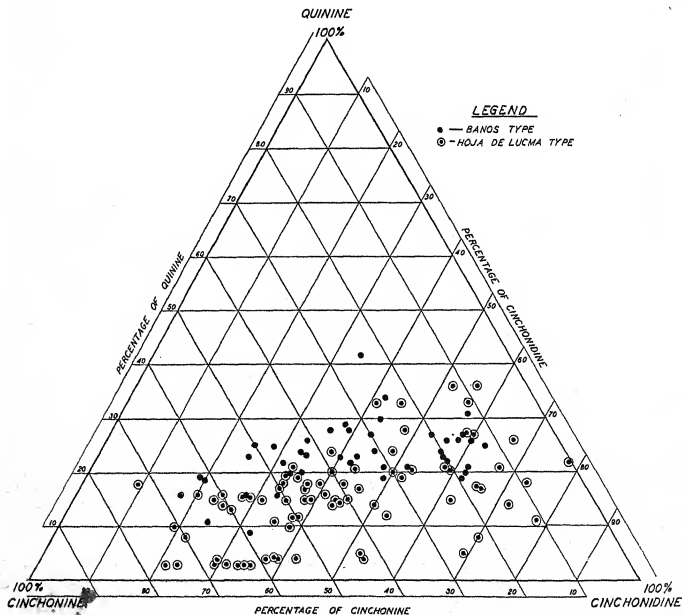
FIG. 6.—Comparison of Hoja de Lucma and Baños types of *C. officinalis*

the Hoja de Lucma, the proportions of the three alkaloids are essentially the same.

Earlier in this paper, the similarity of the proportionate amounts of the alkaloids of the Bolivian Calisaya and Ecuadoran Costrona Fina types of *C. officinalis* was pointed out. As may be seen from figure 7, both are predominantly quinine-formers, and scatter patterns tend to overlap considerably, in spite of the fact that the Calisaya contains nearly twice the total crystallizable alkaloids found in samples of Ecuadoran Costrona Fina.

It may be noted that cinchonine was entirely absent in many of the Ecuadoran Costrona Fina samples, while in every Calisaya sample at least a small proportion was present. Both can be classified as quinine barks, in marked contrast with the other types of cinchona shown in the preceding two diagrams.

The data in the diagrams already presented have been taken principally from commercial samples. As such, they show the general characteristics of the species and type but do not present so complete a picture of genetic variation as may be
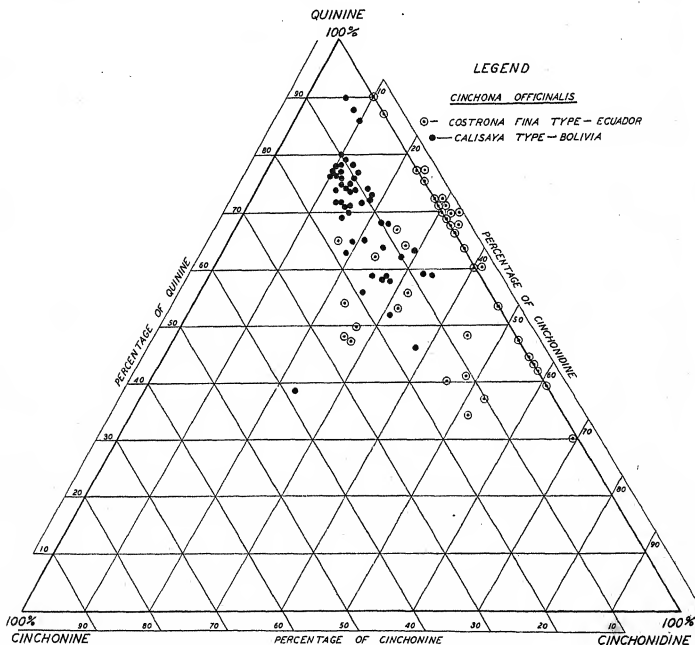
Fig. 7.—Comparison of Bolivian Calisaya with Ecuadoran Costrona Fina types of *C. officinalis*

obtained from a study of individual tree samples. Variation of the latter type is shown in figure 8. In this chart are presented scatter diagrams from samples of the fifty-seven individual trees of *C. pitayensis* sampled in Ecuador and from sixty of the Ledgeriana trees sampled at the Punizas planting in Peru. The twenty Ledgeriana trees containing appreciable amounts of quinidine were not included, as no practical way has been found to plot four variables simultaneously.

The *C. pitayensis* points occur in two distinct zones in figure 8. A group of nine of the fifty-seven samples containing nothing but cinchonine, and less than 2% total crystallizable alkaloids, rests at the cinchonine corner of the triangle. The remaining forty-eight samples occupy almost exactly the same zone of the chart as occupied by the commercial field samples shown in figure 4. The cinchonine samples seem definitely apart from the *C. pitayensis* type and might represent trees of another species, such as *C. pubescens* Bofuda, known to occur at 2000-feet lower elevation on the same mountain. On the other hand, the Bofuda
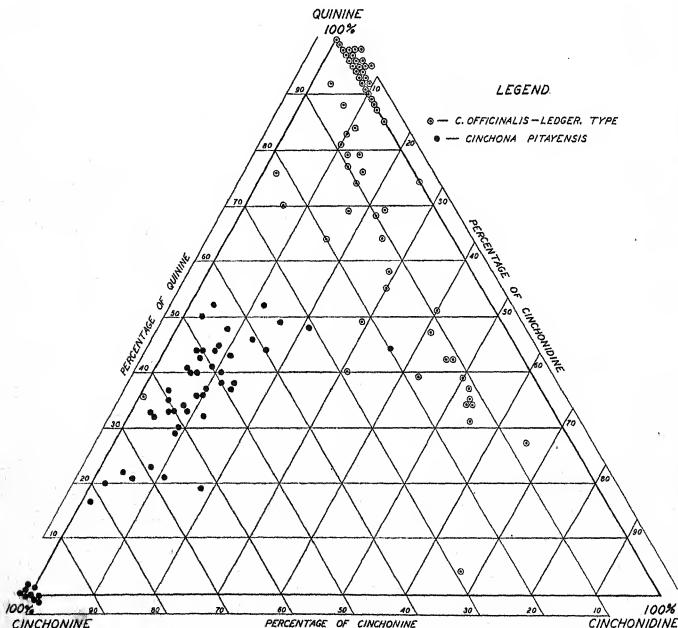
Fig. 8.—Alkaloid ratio pattern of individual tree populations of *C. pitayensis* and cultivated Ledgeriana trees of *C. officinalis*.

type is so distinct from *C. pitayensis* that it could hardly have been mistaken at the time leafy cuttings were taken from each tree for propagation. A possibility exists that these cinchonine trees may be the result of segregation of a supposedly pure species, or else the result of the production of hybrid seed many years ago by the pollination of the *C. pitayensis* trees by *C. pubescens* pollen carried up the mountain several miles by insects or wind.

The fact that Ledgeriana is primarily a quinine-producer is well illustrated in figure 8, which shows a heavy cluster of points above the 85% quinine level. Most of the remaining points occupy the zone containing in figure 7 most of the Bolivian Calisaya samples. The scatter pattern for this Ledgeriana population may well represent the segregation of seedlings derived from plants originally selected in Java for quinine production. The few trees of low quinine and high cinchonine or cinchonidine content may be taken to represent extremes in genetic variability and reversion to a parent type of very different alkaloid-producing characteristics. The position of the points was not found to be related to leaf type, tree age, or any other discernible characteristic. It is of interest that the points not representing trees of predominant (above 80%) quinine production occur in almost the same zone of the chart as samples of Bolivian Calisaya, reported to be the

parent material from which Ledgeriana was selected (6).

Summary

1. Data are presented describing the geographic distribution and showing the approximate composition of the principal commercial cinchona barks of Ecuador, together with comparative composition of other American barks.

2. While the alkaloid quinine is often thought of as the common constituent of cinchona bark, the three additional alkaloids—quinidine, cinchonine, and cinchonidine—are commonly present. The latter two are often present in Ecuadoran barks in amounts greatly exceeding quinine.

3. A study has been made of the variability in composition of Ecuadoran and other American cinchona barks in order to determine their value as breeding material or as sources for the selection and development of clones of high alkaloid-producing capacity.

4. Alkaloid ratio diagrams for the principal cinchona types are presented to show the relative composition of a bark with respect to its alkaloid constituents. These diagrams appear to offer a simple graphic method of presenting genetic variability within a population and may be used as an aid in variety or species delimitation.

ESTACIÓN EXPERIMENTAL DEL ECUADOR AND
FOREIGN ECONOMIC ADMINISTRATION
QUITO, ECUADOR

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HISTOLOGICAL REACTIONS OF BEAN PLANTS TO CERTAIN OF THE SUBSTITUTED PHENOXY COMPOUNDS[1]

J. M. BEAL

Introduction

This paper is one of a series, begun in 1936 (6), dealing with the histological reactions of plants to various growth-regulating substances. The reactions of a number of plants have been described, but no detailed studies have been reported on the histological reactions of any plant to applications of the substituted phenoxy compounds. The gross telemorphic effects of certain of these compounds have been recorded in earlier papers (1, 2). The results there reported accorded with some of those previously given by ZIMMERMAN (10) and by ZIMMERMAN and HITCHCOCK (11). Strikingly different responses or effects have been induced by different phenoxy compounds. In addition, it has been shown (2) that the carrier in which a specific compound is applied may profoundly influence the character and degree of the response. It was demonstrated, for example, that 2-chlorophenoxyacetic acid was effective when applied in Carbowax but largely ineffective in lanolin. An almost completely opposite effect resulted from the application of 4-chlorophenoxyacetic acid in either of the two carriers. Both carriers were almost equally effective for 2,4-di- and 2,4,5-trichlorophenoxyacetic acids, but with somewhat more rapid growth responses and form changes when Carbowax was the carrier. When either of the latter two compounds was applied at a 1% concentration in

Carbowax, the majority of the treated plants were dead within 3 weeks. The surviving plants recovered slowly and then developed chiefly small misshapen leaves. Although slightly fewer plants were killed when lanolin was the carrier, there was little other difference in the responses.

Because the form changes and growth responses had been so pronounced from the application of 1% mixtures at the bases of the blades of the nearly full-sized heart-shaped leaves of the bean, it was decided to conduct further experiments and apply the same compounds at 0.5% concentration. Three additional series of beans have been grown in flats, under conditions which simulated as closely as possible those of the earlier experiments, and treated at different seasons. The first was started on March 1, 1945, and treated on March 13. Temperatures (although fairly cool) and light conditions were favorable to growth at the time. These plants grew well but responded more slowly than those to which 1% mixtures had been applied at approximately the same period the preceding year, when light and temperature conditions were similar. A second series was planted on June 10 and treated on June 21, when the day temperatures in the greenhouse were higher than during March and April. The responses were greater than in the first series but were still somewhat slower than following application of the 1% mixtures. A third series planted on July 20 was treated on July 28. Just

before and immediately after treatment of this series, the outside temperatures exceeded 90° F. during the day and the relative humidity was high. As a consequence, the Carbowax mixtures absorbed water and liquefied, often spreading along the surface of the leaf or down the petiole. The responses in this series were as rapid and as pronounced as they had been with the 1% mixtures, and approximately the same percentage of plants were killed. In general, the growth effects were essentially similar to those previously reported (2), with the least responses being induced by applications of 2-chlorophenoxyacetic acid.

Recently, 2,4-dichlorophenoxyacetic acid has come into prominence as a differential herbicide (3, 7, 8, 9), promising to become highly important for this purpose. Only one report has appeared which deals with the histological changes resulting from application of this substance. TUKEY et al. (9) treated bindweed and sow thistle with aqueous sprays of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. in 0.5% Carbowax 1500, applied during midsummer while the plants were growing vigorously. This resulted in greatly increased cell division in all cambial zones and phloem regions of the stem and rhizome of bindweed, with enlargement and rupture of cortical cells of the rhizome. The root responded similarly but more slowly. In the rhizome of sow thistle, enlargement and tearing of cortical cells was frequent, accompanied by rupture of the periderm. The cambial zone and phloem regions showed disorganized, large-scale cell division. The investigators suggest that the increase in meristematic activity indicates an increase in the rate of respiration, which would deplete the food reserves.

For the present histological studies, usually the entire second internode (seldom exceeding $\frac{1}{4}$ inch in length), a segment of the first internode just below the second node, and a segment of the hypocotyl just below the cotyledonary node were fixed in Navashin's solution, handled according to the tertiary butyl-alcohol paraffin method, and sectioned at 10μ.

Observations

As reported previously (2), the place or region of response is highly variable, not only for the different phenoxy compounds but also for any one of them. Usually little indication of cell activity as a result of treatment is evident in any portion of the stem until approximately 48 hours have elapsed (figs. 2B, 3A, B). Sometimes, however, activity begins earlier in the second internode (fig. 2A), although this is not the general occurrence. The time required is not surprising when it is recalled that the point of application of the mixture is $1\frac{1}{2}$–2 inches distant from the top of the first internode and that the chemical, or some derivative from it, must travel this distance and then up or down the stem before noticeable effects can be produced on the cells above and below the second node.

Although most of the present studies have been made on the first internode, sections from the second internode and the hypocotyl have also been investigated and photographed.

At the time of treatment, the second internode had scarcely begun to elongate, being in most cases only 3–5 mm. in length. The trifoliate leaf was as yet unexpanded. Transections show fascicular cambium, however, which has begun to cut off secondary xylem and phloem, but the interfascicular cambium is not complete (fig. 1A).

The second internode was usually little

affected by applications of 2-chlorophenoxyacetic acid, elongation apparently occurring normally with usually no swelling. Following application of 4-chlorophenoxyacetic acid, however, the second internode became much swollen, pale yellow in color, and failed to elongate in approximately one-third of the plants. Death of these swollen internodes usually occurred in less than 3 weeks following treatment, but lateral shoots commonly developed on these plants from the buds in the axils of the heart-shaped leaves, as well as occasionally from those in the axils of the cotyledons. The development of these shoots was slow, and they produced small, narrow, and usually rolled leaflets (2). As a result of cell proliferation, however, the internode frequently becomes swollen in all the tissues except the epidermis, localized areas of the outer cortex, and perhaps the pericycle (figs. 2A, 4A). The inner cortex, the endodermis, phloem and xylem parenchyma, cambium, ray parenchyma, and pith all show marked activity, with the greatest activity in the cambium, phloem, and ray parenchyma. It has not been possible with the sections available to determine the degree of activity in the pericycle, but it appears unresponsive.

The tissue responses shown in figure 4A are similar to those of figure 14 in the paper by KRAUS, BROWN, and HAMNER (6), which deals with the effects of indoleacetic acid applied to the distal end of decapitated second internodes of bean. One rather striking difference is that root primordia fail to develop in the second internodes of any of the plants following application of the phenoxy compounds at the bases of the blades of the heart-shaped leaves (fig. 10), even after as long as 20 days (fig. 13). It should be pointed out that the degree of maturity of the second internode at the time of treat-

ment is different in the two types of experiments. In the plants to which the compounds are applied at the base of the heart-shaped leaves, the chemical—or some derivative from it—must travel down the petiole and then upward into the second internode before it can incite reaction there. In experiments with decapitated plants, the most striking responses have generally occurred just below the place of application of the chemicals. This may have some bearing on the development or the failure of development of roots following the two methods of application.

The second internode seldom elongates appreciably following treatment with 2,4-di- or 2,4,5-trichlorophenoxyacetic acids but commonly develops the same type of swelling as that resulting from 4-chlorophenoxyacetic acid. Microscopic sections show the same tissues activated and the rate of development to be almost identical from the three compounds.

The tissue responses in the first internode, while much more general and pronounced following applications of 2,4-di- and 2,4,5-trichlorophenoxyacetic acids, were nevertheless similar from all the compounds employed, with the least responses induced by the 4-chlorophenoxyacetic acid. Little indication of increased meristematic activity was evident in the first internode, as was usually true of the second, until approximately 48 hours after treatment (figs. 2B, 3A, B). These early responses are limited to division of the cells of the endodermis, cambium, and medullary rays, with no signs of activation of cells in the other tissues. The early divisions are all in a tangential plane; some of the later ones are radial. Comparison of these figures with figure 1B (from a section of a control plant fixed at the time of treatment)
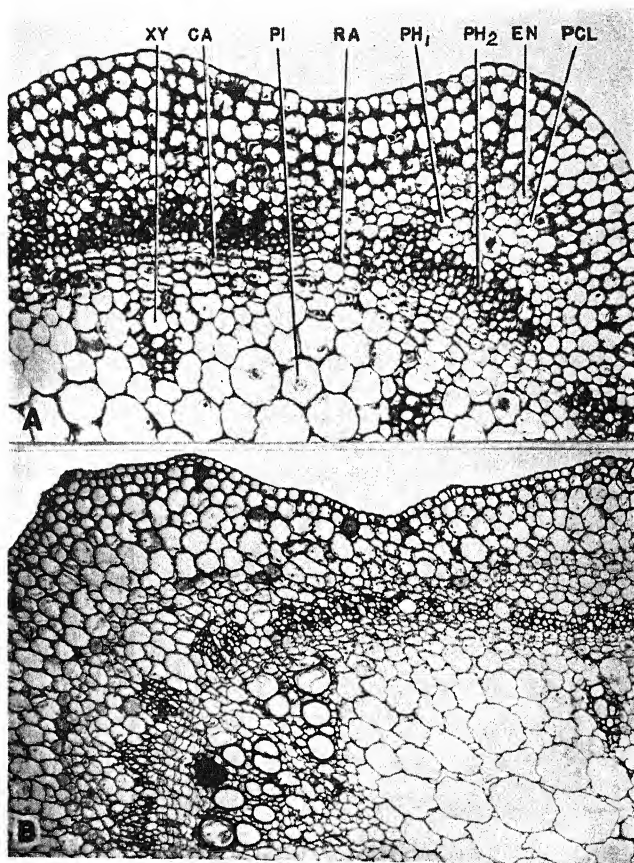
FIG. 1.—A, transection of second internode of bean at time of treatments, showing various tissues and stage of development. (xy, xylem; ca, cambium; pi, pith; ra, ray; phₗ, primary phloem; ph₂, secondary phloem; en, endodermis; pcl, pericycle.) B, first internode of same plant, showing continuous ring of cambium; some derivatives of cambium essentially mature.
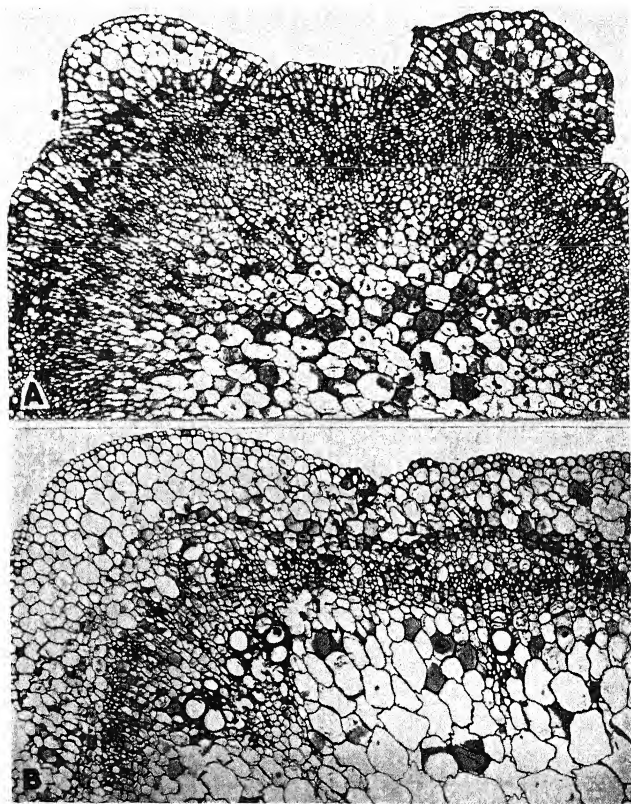
FIG. 2.—Forty-eight hours after treatment with 2,4-dichlorophenoxyacetic acid in Carbowax: *A*, second internode, showing marked activity in all tissues except epidermis, possibly pericycle, and portions of outer cortex. *B*, first internode, showing activity only in endodermis, cambium, phloem, and ray parenchyma; principal activity in endodermis and cambium.
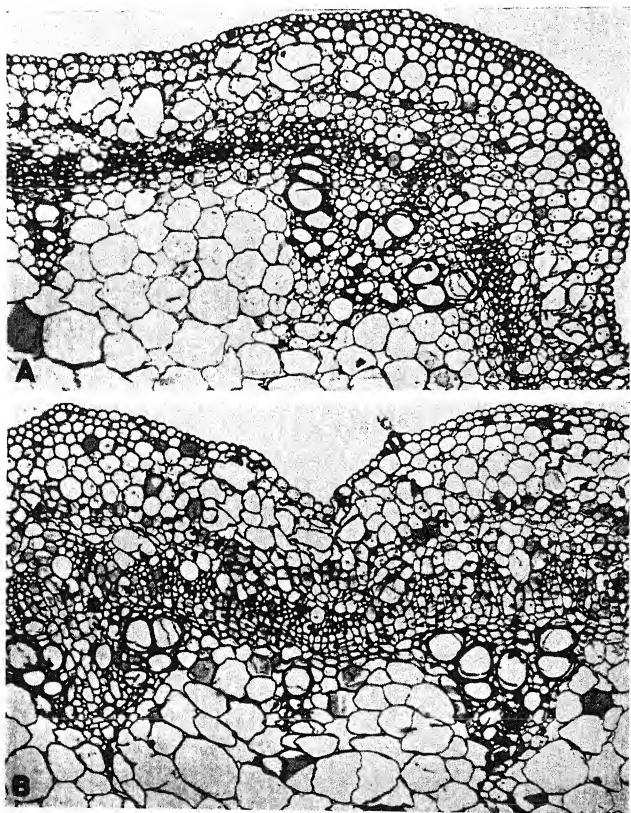
FIG. 3.—First internode 48 hours after treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax, showing practically no induced activity; and *B*, in lanolin, showing activity beginning in endodermis, cambium, and ray parenchyma, with slight activity in phloem parenchyma. Pericycle and pith apparently unaffected.
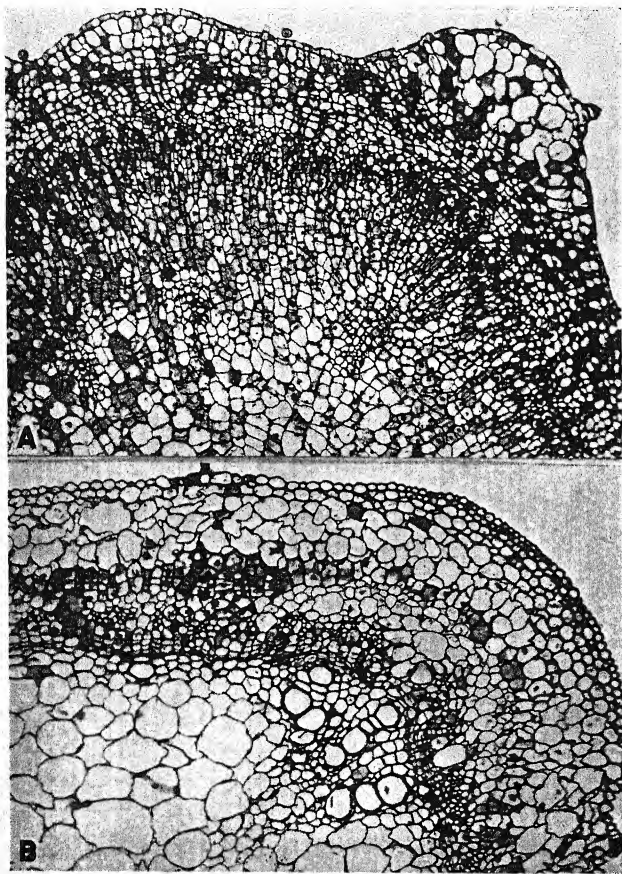
Fig. 4.—Seventy-two hours after treatment: *A*, second internode after treatment with 4-chlorophenoxy-acetic acid in lanolin. All tissues except epidermis and pericycle have been active; similar to fig. 2*A* but responses now more extensive. *B*, first internode after treatment with 2,4-dichlorophenoxyacetic acid in Carbo-wax. Activity principally in endodermis, cambium, and ray parenchyma. Pericycle and pith unaffected.
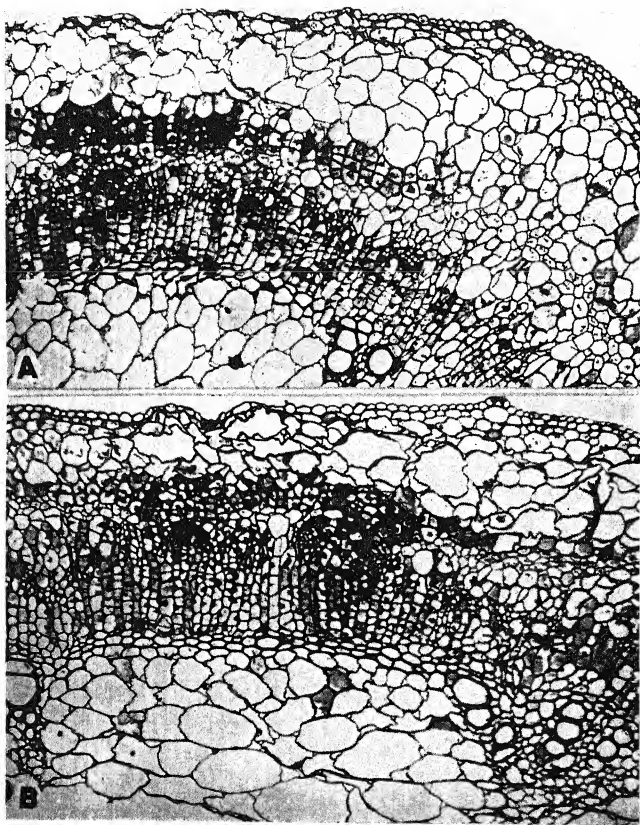
Fig. 5.—First internode 72 hours after treatment with 2,4,5-trichlorophenoxyacetic acid: A, in Carbowax; and B, in lanolin. Responses similar in both, with greatly increased activity in endodermis, cambium, phloem, and ray parenchyma.
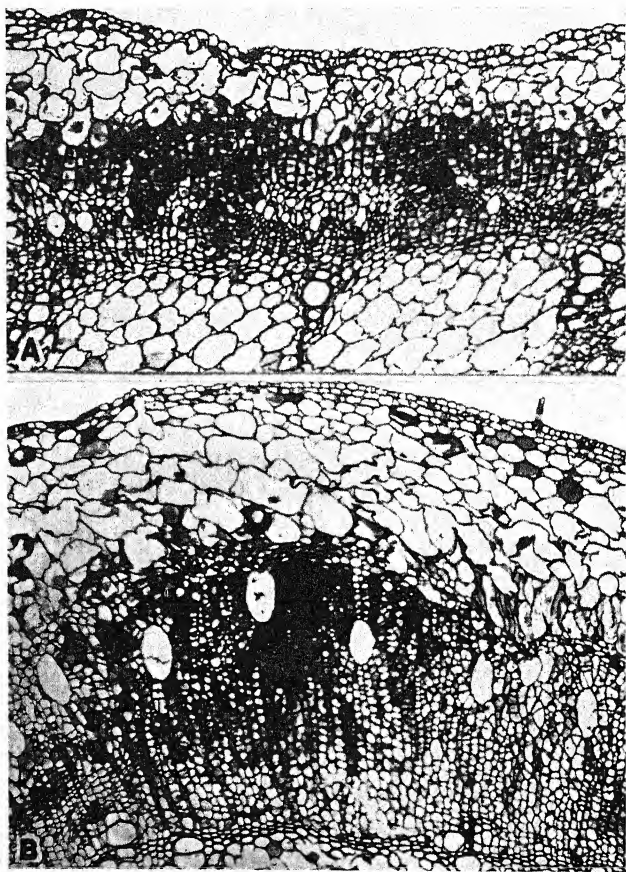
Fig. 6.—*A*, first internode 80 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. Principal activity in endodermis and ray parenchyma, with less activity in cambium and phloem parenchyma. *B*, upper portion of hypocotyl 96 hours after treatment with 2,4-dichlorophenoxyacetic acid in lanolin, showing root primordium derived chiefly from cambium, phloem, and ray parenchyma. Endodermis showing little or no activity.
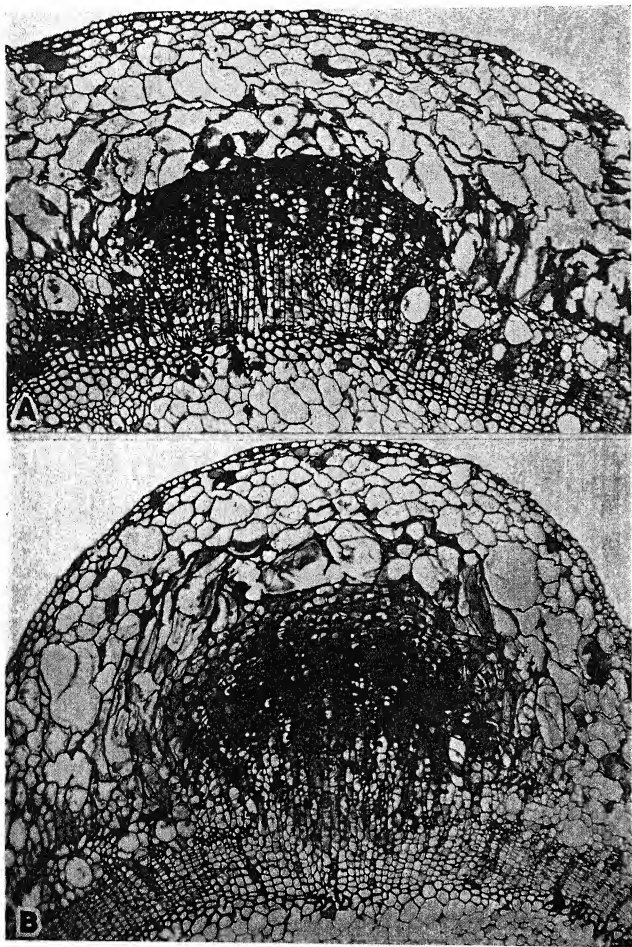
Fig. 7.—Upper portion of hypocotyl: *A*, 96 hours after treatment with 2,4,5-trichlorophenoxyacetic acid in lanolin; and *B*, 144 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. Root primordia organized chiefly from phloem and ray parenchyma, with less activity in cambium. Endodermal and some of inner cortical cells enlarged and crushed over end of root primordium.
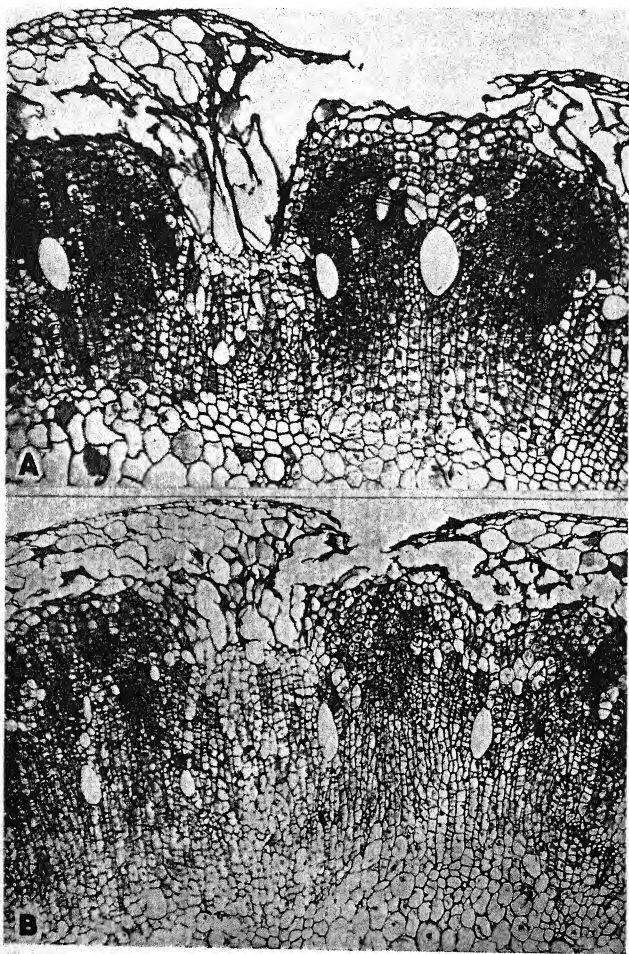
FIG. 8.—First internode 120 hours after treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax; and *B*, in lanolin. Same tissues active as in fig. 6*B*.
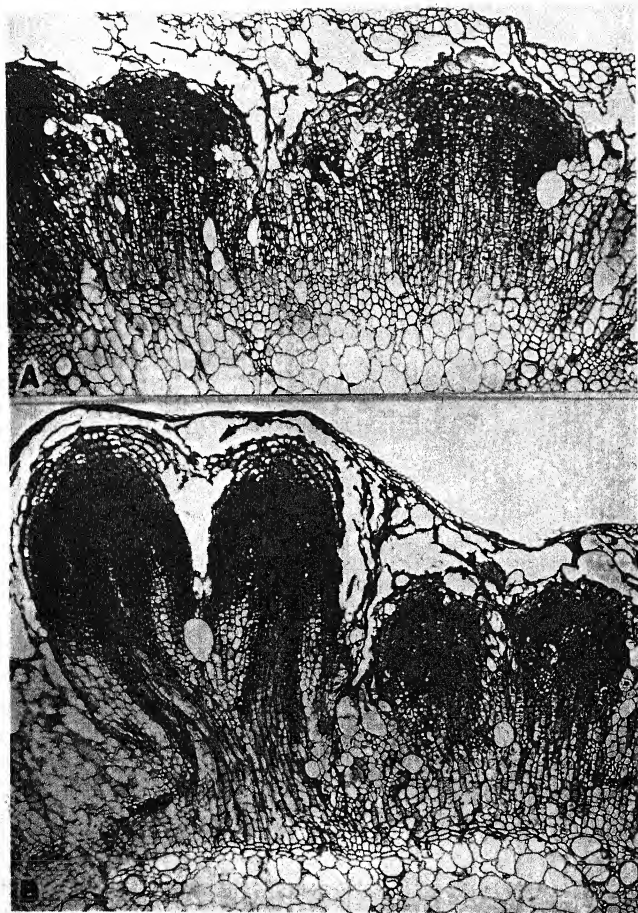
Fig. 9.—*A*, first internode 120 hours after treatment with 2,4,5-trichlorophenoxyacetic acid in Carbowax. Tissues activated and responses similar to those in fig. 8*A*, *B*. *B*, first internode 192 hours after treatment with same compound applied in lanolin. Central core of adventitious roots derived from ray, outer portions from phloem, and tips capped with primary phloem and its derivatives. Endodermis and cortex chiefly crushed and dead.
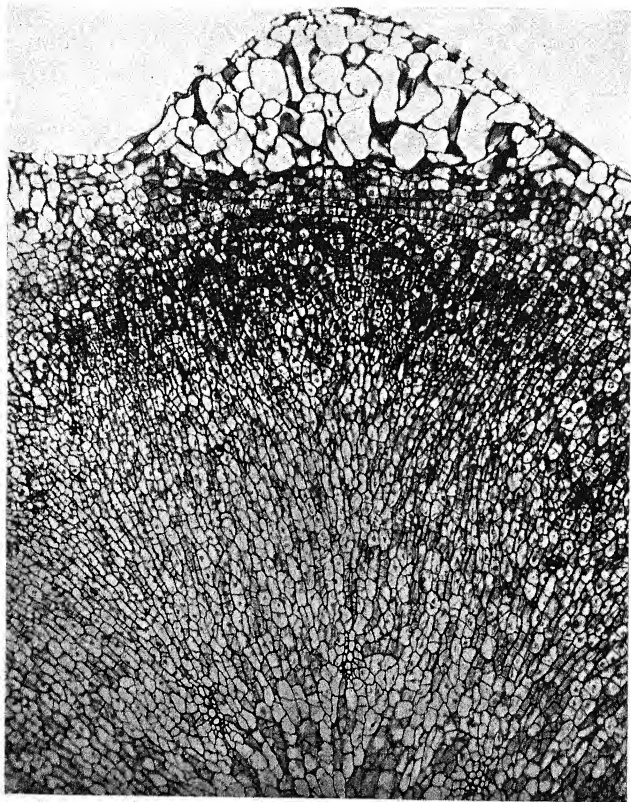
FIG. 10.—Second internode just above second node 144 hours after treatment with 4-chlorophenoxyacetic acid in lanolin, showing marked activity in all tissues except epidermis (and possibly pericycle). Cambium especially active.
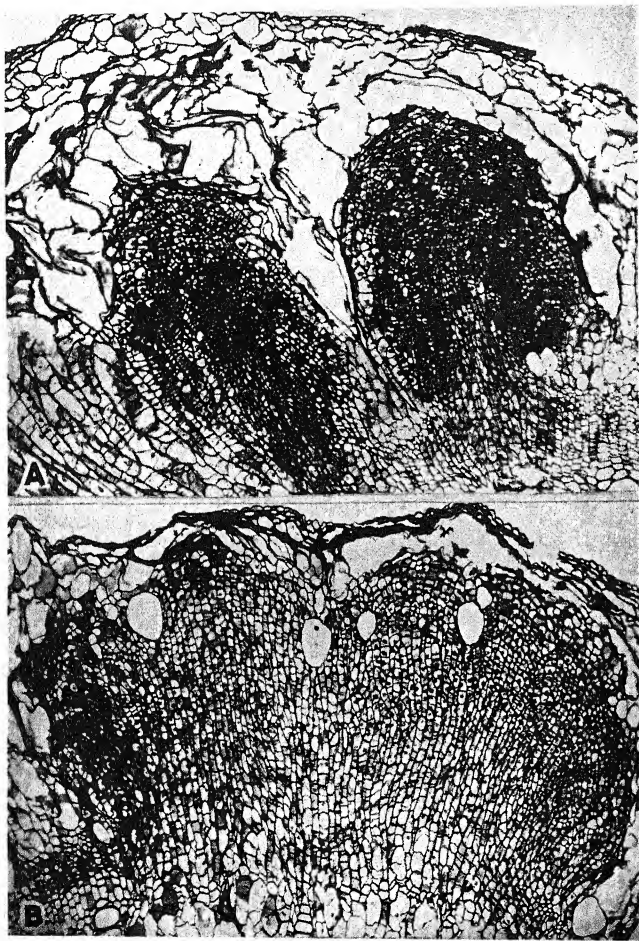
FIG. 11.—First internode 240 hours after treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax; and *B*, in lanolin. Activities similar to but more advanced than in fig. 8*A*, *B*. Epidermis and cortex mostly dead and broken over root primordia.

brings out certain striking differences. In the control (fig. 1*B*), while a complete ring of cambium is present and an appreciable amount of secondary phloem and xylem have differentiated, the endodermis, ray, and phloem parenchyma show no unusual activation (figs. 2*B*, 3*A*, *B*).

At 72–80 hours after treatment the rate of division increases in the endodermis, cambium, and ray tissues, with some divisions having taken place in the phloem parenchyma (figs. 4*B*, 5*A*, *B*, 6*A*). The most highly reactive tissues are the ray and phloem parenchyma, however, and it is principally from them
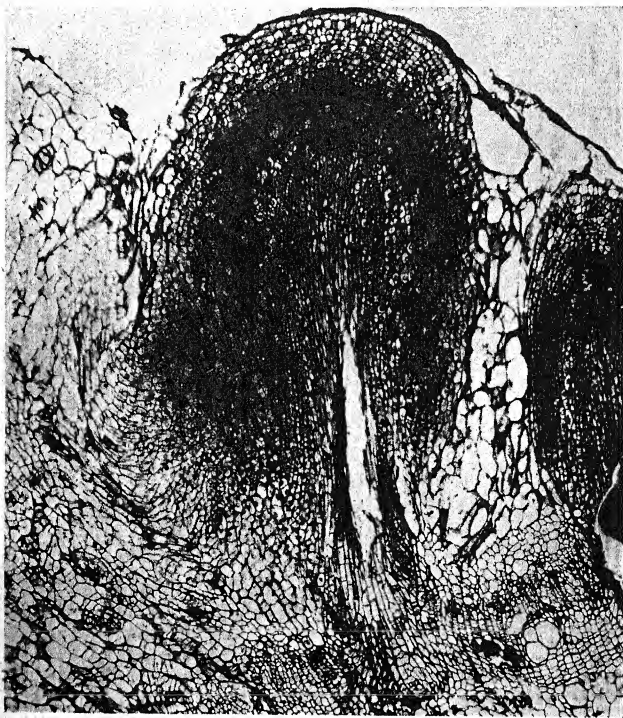
Fig. 12.—First internode 480 hours after treatment with 4-chlorophenoxyacetic acid in Carbowax. Relation of roots to rays similar to that in fig. 9*B*. Cortical tissues mostly dead and crushed.

and their derivatives that the root primordia originate. Concurrently, the cambium, both fascicular and interfascicular, becomes active. The pericycle appears to be inactive and not involved in any of these changes.

At 120 hours, root primordia begin to show definite organization (figs. 8*A*, *B*, 9*A*). In some stems the endodermis fails to become active or there are so few divisions that it plays no significant part in root formation. And while the cambium and its derivatives continue to divide, they contribute less to the formation of the root primordia than do the ray and phloem parenchyma. Cells of the pericycle have in general remained inactive,

unlike *Iresine* (5) and *Mirabilis* (4), in both of which they are highly responsive. Well-developed roots are present in some stems at 192 hours after treatment (fig. 9*B*), although in most stems such roots are not present until 10 days (fig. 11*A*, *B*) or more have elapsed (fig. 12). In these latter figures the epidermis and outer cortical and endodermal cells are mostly dead and collapsed. Perhaps a few derivatives of the endodermis are still alive over the distal ends of the root primordia, but most of this tissue consists of primary phloem. The induced roots originate in the pith rays and may occur in one or more of them.

Activity in the hypocotyl, which re-
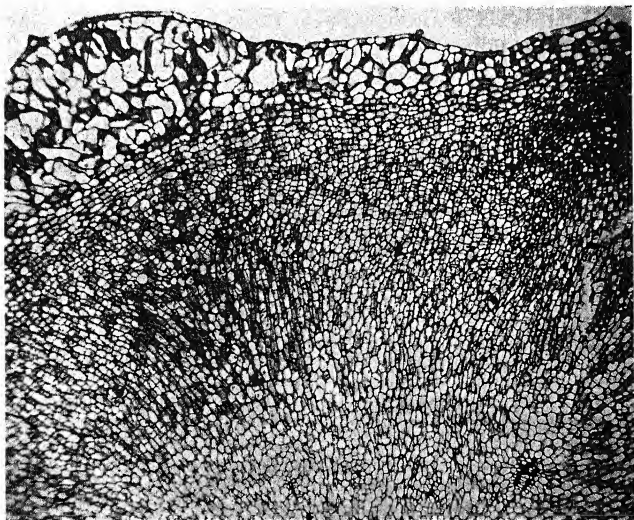
FIG. 13.—Second internode just above node of heart-shaped leaf 480 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. All tissues, especially cambium, have proliferated actively except epidermis, pericycle, and localized areas of outer cortex, but no roots have differentiated.

sults in swelling or in root formation, or both, is common following treatment with 2,4-di- and 2,4,5-trichlorophenoxy-acetic acids. The swelling and subsequent root development commonly involve most of the length of the hypocotyl. And although comparatively few plants show responses in the hypocotyls to applications of 2- and 4-chlorophenoxyacetic acids, and then chiefly in localized areas, the histological responses resulting from the four chemicals are essentially identical and involve the same tissues and tissue systems (figs. 6B, 7A, B). The greatest activity occurs in the ray and phloem parenchyma, with somewhat less in the cambium. The pericycle is inactive, or only slightly active, and plays a very minor role in root development. Root production is largely restricted to the angles of the hypocotyl distal to the protoxylem points of the main or primary vascular bundles.

From an examination of the foregoing figures it is evident that, when the substituted phenoxy compounds are applied at the bases of the heart-shaped leaves of the bean, the tissue responses in the second internode are strikingly similar to those induced by a number of other compounds when such compounds are applied to the cut surface of the young second internode which has been decapitated just below the first trifoliate leaf. Surprisingly, however, no roots developed in this internode following application of the phenoxy compounds to the bases of the heart-shaped leaves, even after periods as long as 20 days following application.

In the first internode and hypocotyl the responses are much more restricted than in the second internode. In the first two of these, generally only the endodermis, cambium, phloem, and ray parenchyma respond. From the cambium, phloem, and ray parenchyma, especially the phloem and ray parenchyma, adventitious roots develop in abundance. Perhaps the greater maturity of the cells in the tissues of the first internode and hypocotyl as compared with the same tissues in the second internode at the time of treatment is, in part at least, responsible for the difference in responses.

Summary

1. Four substituted phenoxy compounds have been applied at the bases of the nearly full-sized heart-shaped leaves of the bean at 0.5% concentration, using both Carbowax 1500 and lanolin as carriers. Lanolin was ineffective as a carrier for 2-chlorophenoxyacetic acid and Carbowax was ineffective for 4-chlorophenoxyacetic acid. The gross responses of the plants to the weaker concentrations were almost identical with those reported previously (2).

2. Histological studies were made of material collected and preserved at 12-hour intervals up to a total of 480 hours. Certain marked differences in responses were observed as between the second internode on the one hand and the first internode on the other. There were also differences in the degree of responses of the plants to the four compounds, although 2,4-di- and 2,4,5-trichlorophenoxyacetic acids resulted in strikingly similar responses.

3. The second internode showed little or no histological response to 2-chlorophenoxyacetic acid. When treated with the three other compounds it became much swollen and generally failed to elongate appreciably in a high percentage of the plants. With the exception of the epidermis, possibly the pericycle,

and limited portions of the outer cortex, all the other tissues responded actively; but no adventitious roots developed, even after 20 days following treatment.

4. The histological responses in the first internode and hypocotyl were strik-ingly similar from all four compounds, but—in contrast to the second internode —only the endodermis, cambium, phloem, and ray parenchyma were activated. Roots developed in abundance.

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RELATIONSHIP OF PHOTOPERIOD AND NITROGEN NUTRITION
TO INITIATION OF FLOWER PRIMORDIA
IN SOYBEAN VARIETIES

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Introduction

Soybean varieties are known to differ in the degree to which they are controlled by photoperiod (2,7). Some varieties flower only when subjected to short photoperiods, while others flower even though they receive very long ones. A number of varieties have been reported as frequently showing significantly increased yield of forage and seed when they are grown on soils high in fertility (with respect to mineral elements) as compared with those which are low (4, 10). With other varieties the yield produced at a low level of fertility is asserted to be more frequently than not equal to that produced at a high level (5, 15). Such a lack of response to variations in soil fertility could perhaps result from the action of some factor of the plant or its environment that might be limiting. An unusual nutritional response in yield is occasionally reported when varieties that show the latter behavior and those that show the former are grown together under different levels of fertility. In this case the yield of one variety will significantly exceed that of another when they are grown at a high fertility level, while at a low level the relative yield of the two varieties is reversed (5, 15).

In previous studies of the influence of various levels of fertility upon yield of different varieties of soybean, no attention has been given to initiation of flower

primordia. It is of interest to know to what extent the growth of such varieties is affected by photoperiod and to determine whether or not differences in time and place of initiation of flower primordia are in any way correlated with the yield differences observed when plants are supplied variable amounts of nitrogen. The present study considers the initiation of flower primordia and expression of other morphological characteristics for plants of four varieties of soybean. Two of the varieties are reported to show increased yields with increased level of fertility, while the yields of the other two are said to be unchanged by different degrees of fertility (4, 5, 9, 15).

Material and methods

Each experiment was concerned with only two of the four varieties of soybean, Morse and Virginia being used in some experiments and Lincoln and T-48 in others. The first two varieties have been in cultivation for many years and the last two were recently developed at the Illinois Agricultural Experiment Station, Urbana, Illinois.[4] The yields of Morse and Lincoln—but not those of Virginia and T-48—are reported to be influenced by fertility level (9, 15).

Plants were grown both in the greenhouse and in controlled environment rooms (13). In two preliminary experi-

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ments, designed to study the photoperiodic requirements of these particular varieties, the plants were grown in soil in the greenhouse. In the other three experiments the plants were grown in sand cultures in either the greenhouse or control rooms, and with various lengths of photoperiod and various amounts of nitrogen added as sodium nitrate. The variations in sodium concentration were not considered of importance, since soybean is a nonaccumulator of this element (3).

Five nutrient solutions (table 1) were employed in the nutrition experiments. All were complete solutions and varied in amount of nitrogen. Solutions supplying a low, intermediate, and high level of nitrogen, respectively, were used in each experiment. Solution 4 (table 1), supplying the intermediate level of nitrogen at rate of 52 p.p.m., was previously developed for growing Biloxi soybean in sand culture, and an apparently normal growth of plants was obtained when it was employed in these experiments. Minor elements were supplied by a modified Hoagland solution (8). Solutions were applied daily in quantities sufficient to provide thorough flushing of the pots.

The plants grown in control rooms received light from a combined carbon-arc–Mazda source of approximately 1200 foot-candles at the surface of the pots (14). Those grown in the greenhouse received photoperiods both longer and shorter than those occurring naturally. In the former case supplemental light of approximately 50 foot-candles from 100-watt incandescent filament bulbs was employed to extend the natural photoperiod; and in the latter the plants were covered with double-thickness, black sateen cloth or were moved into

dark chambers after they had received the required photoperiod.

Throughout the course of each experiment, the position of the first-flower node,[5] the number of nodes at which flower primordia were formed, the total nodes in the main axis, and the presence or absence of terminal inflorescences were determined for plants of the various treatments by microscopic observation. The t-test (17) was employed to measure the significance of differences occurring between plants of the various treatments. Differences sig-

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS

SOLUTION NO.	MOLAR CONCENTRATION OF C.P. SALTS				N (P.P.M.)
	$MgSO_4 \cdot 7H_2O$	$CaCl_2 \cdot 2H_2O$	KH_2PO_4	$NaNO_3$	
1........	0.0058	0.0030	0.0015	0.0010	14
2........	.0058	.0030	.0015	.0015	20
3........	.0058	.0030	.0015	.0018	26
4........	.0058	.0030	.0015	.0037	52
5........	0.0058	0.0030	0.0015	0.0075	105

nificant at the 1% and 5% levels are reported as highly significant and significant, respectively.

Experimentation and results

EXPERIMENT I

An initial experiment was carried out to determine the effect of various lengths of photoperiod on the formation of flower primordia in the varieties Lincoln and T-48. On March 5, 1943, a planting of seed of each variety was made in the greenhouse in soil in 4-inch pots, 144 pots for a variety. The plants were sub-

[5] By "first-flower node" is meant that node on the primary axis of the plant at which the first flower primordia are formed. The cotyledons are located at node one, the primary leaves at node two, and the first compound leaf at node three.

jected to 20-hour photoperiods from time of germination until March 19. Ten plants of each variety were dissected on the latter date and no flower primordia were present on any plant. Beginning at this time, twenty plants of each variety were subjected to daily photoperiods of 12, 14, 16, and 20 hours, respectively.

On March 27, eight plants of each variety were dissected, and flower pri-

TABLE 2

EFFECT OF PHOTOPERIOD ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF LINCOLN AND T-48 SOYBEAN VARIETIES GROWN IN SOIL IN THE GREENHOUSE. SEED PLANTED MARCH 5 AND DATA COLLECTED APRIL 21, 1943. TOTAL OF TWELVE PLANTS PER LOT

Variety	Photo-period (hours)	No. plants flower-ing	Average node at which first-flower pri-mordia occurred	No. plants with ter-minal in-florescence
Lincoln	12......	12	5.0	0
	14......	12	5.8	0
	16......	12	6.5	0
	20......	12	13.6	0
T-48	12......	12	6.2	12
	14......	12	7.2	12
	16......	12	7.8	12
	20......	2	14.0	0

mordia were present on plants of both varieties on the 12- and 14-hour photoperiods, while none were present on those of the 16- and 20-hour lots. The remaining twelve plants of each variety on 12-, 14-, 16-, and 20-hour photoperiods were dissected on April 21, when the experiment was terminated. Although flower primordia were present on all plants of the 12-, 14-, and 16-hour lots, and on some plants of the 20-hour lots (table 2), nevertheless there were differences between varieties and between photoperiods in the position of the first-flower primordia formed. With the

20-hour photoperiod, however, flower primordia were present on only two of the twelve T-48 plants, while they were present on all twelve of the Lincoln plants. The average position of the first-flower primordia on plants of each of the three shorter photoperiods, respectively, was approximately one node lower for Lincoln than it was for the T-48; with the 20-hour photoperiod this difference between varieties did not occur. Lincoln plants did not form a terminal inflorescence with any of the four photoperiods and were still forming vegetative structures at the apex of the main stem, while T-48 plants formed such an inflorescence when grown on each of the three shorter photoperiods.

EXPERIMENT II

On April 10, 1943, an experiment was started in the greenhouse with Lincoln and T-48 in which the plants were subjected to two different lengths of photoperiod and three different conditions of nitrogen nutrition. Eight treatment blocks were established, each consisting of six rows and ten pots per row. Three successive rows of each block were restricted to a variety. The relative order of varieties was alternated throughout the eight blocks.

Nutrient solutions 3, 4, and 5 (table 1), varying in amount of nitrogen, were employed, a different solution for each row of a variety within a treatment block. Solutions 3, 4, and 5 supplied nitrogen at rates of 25, 52, and 105 p.p.m., respectively, and were first applied immediately after planting of seed on April 10. All plants were maintained on 18-hour photoperiod until April 21, at which time the plants were thinned to one per pot and four of the treatment blocks were started on a 14-hour and the four others on an 18-hour

photoperiod. These treatments were continued until the experiment was terminated on June 15, 1943.

Throughout the experiment several dissections were made of plants from the various treatment lots. The first of these, made on April 21 when the plants were thinned to one per pot, consisted of ten plants of each variety from each of the three nitrogen levels. Each lot of ten plants had developed an average of eight nodes per plant, and no flower primordia were present on any plant. On April 29, three plants of each variety on both durations of photoperiod and the low and high nitrogen treatments were dissected. The plants were obtained from pots that served as guard rows for the various treatment blocks. On the 18-hour photoperiod the only plants with flower primordia were those of Lincoln grown with the low nitrogen treatment. With the 14-hour photoperiod, all plants except those of T-48 on the low nitrogen treatment had flower primordia. A third dissection, identical in size of sample with that of April 29, was made on May 6. Flower primordia were found on all plants except those of T-48 grown with an 18-hour photoperiod.

On May 22, plants from two of the 14-hour photoperiod blocks and two 18-hour blocks were harvested and dissected. The position of the first-flower node was determined for all plants of both photoperiods. The total number of nodes at which flower primordia were formed, and the total number of nodes in the main axis, were obtained only for the plants that had received 18-hour photoperiods (table 3). Similar data could not be obtained with accuracy for the plants on 14 hours because of the compactness of their terminals. The average position of the first-flower primordia for Lincoln and T-48 was at the 5.5 and 6.8 nodes,

respectively, when plants were grown with the intermediate nitrogen treatments on the 14-hour photoperiod. An obviously significant difference between varieties in the location of first-flower primordia resulted when they were grown with this nitrogen treatment and an 18-hour photoperiod. In this case the primordia were developed at nodes 8.0 and 12.8 for plants of Lincoln and T-48, respectively. The same difference also existed for the high and low nitrogen treatments. Since no significant difference occurred among the various nitrogen treatments of either variety for position of the first-flower node when grown on the 14-hour photoperiod, the only data recorded in table 3 are for plants on the 18-hour photoperiod.

The position of the first-flower primordia on plants of T-48 on 18-hour photoperiod was influenced by the composition of the nutrient solution. In this case the difference between lots of the high as compared with those of the intermediate and low nitrogen treatments was highly significant. The greatest difference in position of first-flower node for plants of Lincoln occurred between those grown on the low and those on the high nitrogen treatments, but this difference was not statistically significant.

Highly significant differences in the number of nodes in the main axis that bore flower primordia occurred for plants of T-48 grown with the highest and intermediate nitrogen concentrations as compared with those at the low nitrogen concentration. No significant differences occurred in number of nodes bearing flower primordia for plants of Lincoln on the three nitrogen concentrations. On the other hand, while T-48 plants developed a decreasing number of flower nodes with increasing concentration of nitrogen, Lincoln plants developed a

greater number of flower nodes with the intermediate and high concentrations as compared with the low.

On the 18-hour photoperiods only Lincoln plants displayed a highly significant difference in total nodes. Such difference occurred between plants grown with low as compared with either those at intermediate or those at high nitrogen concentration. In general, with both varieties an increasing number of nodes were developed with increasing amounts of available nitrogen.

factory morphological data could not be obtained by the microscopic methods employed.

With the 18-hour photoperiod all the T-48 plants at each of the three concentrations of nitrogen had initiated a terminal inflorescence. Based on a total of twenty plants, the average node position above which terminal inflorescences were formed was 20.7, 23.4, and 24.1 for plants grown with the low, intermediate, and high concentrations, respectively. The Lincoln plants were just beginning to

TABLE 3

EFFECT OF NITROGEN CONCENTRATION ON EXPRESSION OF CERTAIN MORPHO-
LOGICAL CHARACTERISTICS OF LINCOLN AND T-48 SOYBEAN VARIETIES
GROWN WITH SAND CULTURE IN THE GREENHOUSE ON 18-HOUR PHOTOPERIOD.
SEED PLANTED APRIL 10 AND DATA COLLECTED MAY 22, 1943. AVERAGE OF
TWENTY PLANTS PER LOT

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Lincoln	26	7.2 ± 1.3	8.9 ± 0.9	$18.9 \pm 0.7*$
	52	8.0 ± 1.3	11.0 ± 1.6	22.1 ± 0.7
	105	9.2 ± 1.1	10.0 ± 1.6	22.4 ± 0.7
T-48	26	12.0 ± 0.3	5.0 ± 0.5	19.4 ± 0.5
	52	12.8 ± 0.4	4.9 ± 0.7	21.0 ± 0.4
	105	$15.6 \pm 0.8*$	$2.5 \pm 0.5*$	21.5 ± 0.8

* Significantly different from other two treatments at 1% level.

The sixty plants of each of the two remaining 18-hour photoperiod blocks were harvested and dissected on June 15, when the experiment was terminated. The main objective of this dissection was to locate the position of the terminal inflorescence for plants of each variety, but it also afforded an opportunity to recheck the position of first-flower primordia as determined by the dissection of May 22. The plants of the remaining 14-hour photoperiod blocks were not dissected, since as early as May 22 the nodes and internodes in the terminals had become so compacted that satis-

form terminal inflorescences. Of a total of twenty plants, one Lincoln plant formed a terminal inflorescence at the low nitrogen level, while eight and nine plants from lots of twenty each had done so at the intermediate and high concentrations, respectively. These inflorescences were formed above the 23.0, 25.5, and 25.7 nodes for plants of the low, intermediate, and high concentrations, respectively.

As was expected, the locations of the first-flower primordia for plants of Lincoln and T-48 were the same as observed on May 22. These primordia were de-

veloped on Lincoln at the 7.2, 8.2, and 9.2 nodes, respectively, for plants grown with the low, intermediate, and high concentrations, while on T-48 plants the first-flower node was at the 11.9, 12.7, and 15.5 node, respectively, with these nitrogen concentrations.

EXPERIMENT III

Plants for this experiment were grown with sand culture in 2-gallon glazed crocks with bottom drains and in a con-

a different solution for eight crocks of each variety. The plants were subjected to an 18-hour photoperiod, beginning with germination of seed. On August 23 the plants were thinned to three per crock, and three plants of a variety on each of the three nitrogen treatments were dissected. The plants had no flower primordia and each had a total of eight nodes.

The 18-hour photoperiod and the three nitrogen treatments were continued until September 25, at which time

TABLE 4

EFFECT OF NITROGEN CONCENTRATION ON EXPRESSION OF CERTAIN MORPHO-
LOGICAL CHARACTERISTICS OF LINCOLN AND T-48 SOYBEAN VARIETIES GROWN
IN CONTROLLED-ENVIRONMENT ROOM WITH SAND CULTURE AND 18-HOUR
PHOTOPERIODS. SEED PLANTED AUGUST 18 AND DATA COLLECTED SEPTEM-
BER 25, 1943. AVERAGE OF TWENTY-FOUR PLANTS PER LOT

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Lincoln	20	9.5±0.7†	3.5±1.1	16.5±1.5*
	52	11.5±0.8	5.9±1.6	21.3±1.1
	105	11.8±0.9	6.0±1.4	21.7±0.9
T-48	20	10.8±0.5†	4.2±1.1	17.9±1.3
	52	12.8±0.6	3.2±1.0	19.7±1.0
	105	13.1±0.8	3.6±1.1	20.1±0.8

* Significantly different from other two treatments at 1% level.

† For T-48, significantly different from other two treatments at 5% level; for Lincoln, significantly different from 105 p.p.m. nitrogen treatment at 5% level.

trolled-environment room maintained at $70° \pm 1°$ F. Seed of Lincoln and T-48 varieties was planted August 18, 1943. The twenty-four crocks of a variety occupied half of each of two opposite, semicircular benches. The blocks of the respective varieties alternated from one bench to the other. The benches were so constructed and oriented with reference to the light source that equal light energies fell at the surface of all pots.

Solutions 2, 4, and 5 (table 1), supplying nitrogen at 20, 52, and 105 p.p.m., respectively, were applied to the plants,

the experiment was terminated and all plants were dissected. The node at which the first-flower primordia occurred, the number of nodes bearing recognizable flower primordia, and the total number of nodes in the main axis were observed for each plant. The data are shown in table 4.

In general, the data indicate trends similar to those observed for plants grown in the greenhouse where comparable photoperiod and nitrogen treatments were employed (experiment II; table 3). Differences significant at the

5% level were observed for node position of first-flower primordia with Lincoln plants grown at the low and the high nitrogen levels. There was no significant difference between plants of any other two treatments. Differences significant at the 5% level occurred for position of first-flower primordia of T-48 plants grown at low in comparison with either intermediate or high nitrogen levels.

With respect to total flower nodes there were no significant differences between plants of either Lincoln or T-48 at the various nitrogen levels. However, as was the case in experiment II, plants of the Lincoln variety had an increasing number of flower nodes with increasing nitrogen concentration, while with T-48 plants the highest number of flower nodes was developed at the lowest nitrogen concentration.

With increasing concentration of nitrogen there was an increasing number of nodes developed on the main axis for both Lincoln and T-48 plants. The difference in number of nodes was highly significant for plants of Lincoln grown with low as compared with either the intermediate or high nitrogen levels. In experiment II (table 3) this same degree of difference was observed only between plants of the low and high nitrogen levels. The difference in total nodes of T-48 plants grown with the various levels of nitrogen was not significant, however. This parallels the results observed for plants of this variety grown in the greenhouse experiment (table 3).

Experiment IV

In this experiment the effects of duration of photoperiod on the initiation of flower primordia and the differentiation of terminal inflorescences were observed for Morse and Virginia varieties. On June 21, 1943, plantings of each variety were made in soil in 4-inch pots in the greenhouse. Plants were thinned to one to a pot, and ten of each variety were subjected to photoperiods of 8, 10, 12, 14, 16, and 18 hours.

Two plants of each variety on each duration of photoperiod were dissected on July 10. Flower primordia were present on all those grown with 8-, 10-, and 12-hour photoperiods. All Virginia plants had developed terminal inflorescences on these photoperiods but the Morse plants had not. Flower primordia were also present on the Virginia plants of the 14-hour photoperiod but terminal inflorescences were not developed. One of the Morse plants on 14-hour photoperiod had developed flower primordia and none of either variety on 16- or 18-hour photoperiod had formed them.

The remaining eight plants of each variety on the various photoperiods were dissected when the experiment was terminated on July 22, and the data are shown in table 5. By this time both varieties had initiated flower primordia at all photoperiods from 8 to 18 hours inclusive, but, as was the case for Lincoln and T-48, the development of first-flower primordia was progressively delayed in both varieties by the longer photoperiods (table 5). Plants of Virginia formed terminal inflorescences when grown on photoperiods up to 14 hours, inclusive. No such inflorescence was developed by any Morse plant when grown with these photoperiods nor by plants of either variety when grown with longer photoperiods. On 8- to 16-hour photoperiods Virginia initiated first-flower primordia at a lower node position than did Morse. This means that flower-bud formation occurred earlier on Virginia than on Morse when the two varieties were grown with short photoperiods. On 18 hours, however, there was little dif-

ference in relative earliness, the node position of first-flower primordia being about the same for the two varieties. Thus varieties may differ in relative earliness when grown with long and short photoperiods.

TABLE 5

EFFECT OF PHOTOPERIOD ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF MORSE AND VIRGINIA SOYBEAN VARIETIES GROWN IN SOIL IN THE GREENHOUSE. SEED PLANTED JUNE 21 AND DATA COLLECTED JULY 22, 1943. TOTAL OF EIGHT PLANTS PER LOT

PHOTO-PERIOD (HOURS)	NO. PLANTS WITH FLOWER PRIMORDIA		AVERAGE NODE AT WHICH FIRST-FLOWER PRIMORDIA OCCURRED		NO. PLANTS WITH TERMINAL INFLORESCENCE	
	Morse	Vir-ginia	Morse	Vir-ginia	Morse	Vir-ginia
8....	8	8	5.0	3.0	o	8
10....	8	8	5.7	3.4	o	8
12....	8	8	5.6	3.6	o	8
14....	8	8	8.5	5.6	o	8
16....	8	8	9.9	8.4	o	o
18....	8	8	14.0	14.5	o	o

EXPERIMENT V

Plants of this experiment were grown in sand cultures in a controlled-environment room and with methods similar to those used in experiment III. Seed of Morse and Virginia were planted on July 15, 1943. Solutions 1, 4, and 5 (table 1) were employed which supplied nitrogen at rates of 14, 52, and 105 p.p.m., respectively. Eight crocks of each variety received the same solution. The plants were subjected to a 15-hour photoperiod immediately following germination, and were thinned to four to a crock on July 24. At this time they were expanding their first compound leaf. Two plants of a variety at each nitrogen level were dissected at this time and no flower primordia were present. All

Morse plants had developed eight nodes, while those of Virginia had 7.6 nodes with the low and intermediate nitrogen levels and 8.0 with the high level. The plants of each variety on each nitrogen level removed at the time of thinning were subjected to chemical analysis. The samples for analysis consisted of the portion of the plant above the primary leaves. Ammonia, nitrate, and soluble nonprotein nitrogen were determined according to methods reported earlier (13). There was no appreciable difference in the ammonia content, and the values are not reported. The data (table 6) indicate that plants of each variety grown with different concentrations of nitrogen contain different percentages of nitrate and soluble nonprotein nitrogen. With both varieties the percentages of nitrate nitrogen for plants with intermediate concentrations were more than double those

TABLE 6

PERCENTAGE OF NITRATE AND SOLUBLE NON-PROTEIN NITROGEN IN MORSE AND VIRGINIA SOYBEAN VARIETIES GROWN WITH SAND CULTURE FOR 9 DAYS IN CONTROLLED-ENVIRONMENT ROOM. PLANTS SUBJECTED TO 15-HOUR PHOTOPERIOD AND VARIOUS CONCENTRATIONS OF NITROGEN. DATA BASED ON FRESH WEIGHT OF TISSUE

Variety	Nitrogen in nutrient solution (p.p.m.)	Nitrate nitrogen (%)	Soluble nonprotein nitrogen (%)
Morse	14........	0.021	0.253
	52........	.044	.272
	105........	.046	.310
Virginia	14........	.022	.166
	52........	.052	.210
	105........	0.057	0.234

for plants grown with low nitrogen concentration. The percentage of nitrate nitrogen in plants supplied intermediate and high nitrogen concentrations were more nearly alike. The percentage of

soluble nonprotein nitrogen of plants increased with increasing amounts of nitrogen in the nutrient solution, but the magnitude of the difference from the low to the high concentration was considerably less than that which occurred for the percentage of nitrate nitrogen. The chemical analysis data indicate that the nitrate and soluble nonprotein nitrogen content of the seedlings reflect the nitrogen content of the nutrient solution after

first-flower primordia of Morse occurred at nodes 8.9 and 9.9 on plants receiving 14 and 105 p.p.m. of nitrogen, respectively. Virginia plants formed their first-flower primordia at 8.1 and 7.8 nodes, respectively, with these nitrogen concentrations.

With respect to total flower-bearing nodes, there was an increasing number formed on plants of both varieties with increasing nitrogen concentration. No

TABLE 7

EFFECT OF NITROGEN CONCENTRATION ON EXPRESSION OF CERTAIN MORPHO-
LOGICAL CHARACTERISTICS OF MORSE AND VIRGINIA SOYBEAN VARIETIES
GROWN IN CONTROLLED-ENVIRONMENT ROOM WITH SAND CULTURE AND 15-
HOUR PHOTOPERIOD. SEED PLANTED JULY 15 AND DATA COLLECTED AUGUST
12, 1943. AVERAGE OF THIRTY-TWO PLANTS PER LOT

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Morse	14	8.9 ± 0.5	2.3 ± 0.7	14.8 ± 0.7
	52	9.7 ± 0.5	3.9 ± 0.8	17.5 ± 0.8†
	105	9.9 ± 0.3	3.9 ± 0.7	17.6 ± 0.7*
Virginia	14	8.1 ± 0.3	2.4 ± 1.0	14.4 ± 0.8
	52	8.0 ± 0.6	5.5 ± 0.9*	17.2 ± 0.7†
	105	7.8 ± 0.4	5.6 ± 1.0*	17.3 ± 0.8†

* Significantly different from 14 p.p.m. nitrogen treatment at 1% level.
† Significantly different from 14 p.p.m. nitrogen treatment at 5% level.

only 9 days of differential nitrogen treatment.

The plants were grown with the 15-hour photoperiod and the various nitrogen treatments until August 12, at which time the experiment was terminated and all plants dissected. The node at which the first-flower primordia occurred, the number of recognizable flower-bearing nodes, and the total nodes in the main axis were observed for each plant. The data are given in table 7.

The various nitrogen treatments did not result in significant differences in position of the node bearing the first-flower primordia in either variety. The

significant differences for total flower nodes occurred for plants of Morse, while highly significant differences occurred for those of Virginia grown on the low in comparison with the intermediate or high nitrogen levels. The difference in total nodes of Morse plants grown with the low nitrogen level and those with the high was highly significant. The difference between the total nodes of the plants grown with low and intermediate nitrogen levels was significant at the 5% level. With Virginia significant differences occurred between plants grown with the low and either the intermediate or the high nitrogen level.

Discussion

BORTHWICK and PARKER (2) report that certain soybean varieties initiate flower primordia only when subjected to short photoperiods, while others initiate such primordia even though the plants receive continuous illumination. With varieties showing the latter response the effect of photoperiod manifests itself through a shift in the position at which the first-flower primordia are formed on the plant. The varieties Morse, Virginia, Lincoln, and T-48 are capable of initiating flower primordia on relatively long photoperiods and may possibly be able to do so with continuous illumination. The first-flower primordia of the varieties were initiated at a slightly higher node position on the plant axis with photoperiods of increasing duration up to 14 hours. With photoperiods of 16 hours, flower primordia were produced at positions considerably higher for each variety than they were with photoperiods of 14 hours, and with further increases in photoperiod these differences became greater. This is interpreted to mean that with daylengths of 16 hours and longer the factor of photoperiod becomes more critical for floral initiation and that slight changes in duration of photoperiod at this level result in marked differences in floral initiation. Although such varieties as Morse, Virginia, Lincoln, T-48, and others (2) can initiate flowers on extremely long photoperiods, the fact that they initiate them at progressively lower node positions with decreasing daylength substantiates the view that these varieties should be regarded as short-day plants.

The data in the literature on the nutrient requirements of soybean for optimum plant development are somewhat conflicting. This appears to be due in part to a factor of varietal adaptability which commonly occurs when varieties are grown on soils of different fertility or in localities in which other factors of the environment may likewise be limiting (11). The more recent field work on soybean nutrition has been carried out in the Midwest cornbelt area. LANG (10), in summarizing recent fertility studies, states that, in general, soybean varieties are adapted to a wide range of soil types and yield better on comparatively infertile acid soils than do any other crop plants in the cornbelt area. However, it is stated that for heavy yields they generally require relatively large quantities of plant nutrients, in comparison with other crop plants such as corn, and show a marked yield response to variations in natural soil productivity and to those treatments that develop soils to a favorable fertility for other crop plants which may follow (10). Based on field experiments, NORMAN (12) reports that soybean can effectively use more nitrogen than is provided through nitrogen fixation in the plant. The results of these workers suggest that some varieties may respond to different levels of fertility while others do not, when they are grown on certain natural daylengths.

POEHLMAN (15) reported that Morse variety in the field outyielded Virginia on soils of high fertility. On the less fertile soils the difference in forage yield between the two varieties diminished, and on the least fertile soils Virginia outyielded Morse. This reversal in variety yield with change in fertility level has also been noted by ETHRIDGE and HELM (5). MORSE (11) likewise reports that Virginia is adapted to soils of low fertility but makes no comparison of its behavior with the Morse variety. In experiments in which plants were grown with sand culture, ALLEN (1) observed that Morse was far superior to Virginia in forage

yields when both were grown with high concentrations of either potassium or magnesium. ETHRIDGE and HELM (5) found Virginia and Wilson varieties superior to Morse and Mikado in yield of both seed and hay when they were grown on Lintonia fine sandy loam soil which was low in fertility. In contrast to Virginia and Wilson, the growth of Morse and Mikado was short and stemmy and their seeds were borne so near the ground that considerable loss occurred in harvesting. Lincoln and T-48 have only recently been grown in the Midwest, and it is reported that the former responds to fertilizer while the latter does not (9).

The present study has considered the response of Lincoln, T-48, Morse, and Virginia to different levels of nitrogen only in terms of the expression of certain morphological characteristics. For this reason no attempt is made to correlate the present experimental results with the yield results of varieties under field conditions. However, certain data have been collected which may furnish a basis for further study of the problem.

The data indicate that although none of the four varieties investigated is prevented from flowering, the initiation of flower primordia in each is delayed by longer photoperiods. As noted earlier, when the factor of photoperiod alone was varied, the greatest differences in position of first-flower primordia occurred when the photoperiods were relatively long. When the factors of photoperiod and nitrogen were both varied in the initial experiments with Lincoln and T-48, the greatest changes in position of first-flower primordia again occurred with the longer daylengths. Similarly, significant differences for both the total nodes and the total number of nodes bearing flower primordia on the main

axis occurred only when plants were grown with long photoperiods. A somewhat similar plant response to the factors of nitrogen nutrition and photoperiod has been noted in onion (16). Bulbing normally takes place only under long photoperiods, but when certain varieties are maintained on photoperiods at or near that critical for bulb formation the plants bulb earlier with low nitrogen concentration than with high. If the plants were to be grown for a sufficiently long period with these daylengths, however, those on low nitrogen would most likely form smaller bulbs. On the other hand, on photoperiods substantially longer than that critical for bulb formation there was no difference in bulb development when the plants were supplied different amounts of nitrogen.

The node position at which first-flower primordia of the soybean varieties were formed varied more in response to nitrogen nutrition when plants received long photoperiods than when they received short ones. The flower-forming stimulus of short photoperiods was so strong that flower primordia were formed when the seedlings were still very small. Floral initiation in these lots occurred before the influence of added nitrogen had become effective, so it is not surprising that variations in nitrogen nutrition were without effect on the position of first-flower primordia on such plants.

With long photoperiods, significant differences in position of first-flower primordia resulting from variations in nitrogen nutrition occurred in Lincoln and T-48 but not in Morse and Virginia. Morse and Virginia received a 15-hour photoperiod instead of an 18-hour one, however, and this may account for the difference in response of the two groups. The formation of first-flower primordia by both Lincoln and T-48 plants was

latest in the lots that received the greatest amount of nitrogen, and although significant differences occurred with both varieties, the differences were greater in T-48 than in Lincoln.

With increasing amounts of nitrogen, Morse and Virginia showed an increasing number of nodes bearing flower primordia. The same trend was shown for Lincoln, but the differences were not significant. On the other hand, T-48 showed the reverse trend. With this variety the effectiveness of the flower-inducing stimulus was evidently partially suppressed in plants at the higher nitrogen level because these plants had produced as many nodes and therefore as many positions for the formation of flower primordia as plants receiving the lower concentration of nitrogen. This result may be correlated in some degree with the fact that this variety is reported to respond less to field applications of fertilizer.

Two general types of development are recognized in soybeans. One is called determinate and the other indeterminate (18). The distinction is mostly concerned with the kind and location of the inflorescence. Development is indeterminate when no terminal inflorescence is formed and determinate when such an inflorescence is formed. The pod-bearing habit is also determined by the development habit of the plant and whether or not terminal inflorescences are formed.

On the basis of mode of pod formation, the determinate and indeterminate types have been described by ETHRIDGE, HELM, and KING (6). With the latter there is "a dense array of pods on the central stem, terminating there in a blunt apex, with a thin dispersal on the lateral branches; and, with the former, a sparse and comparatively even distribution of pods over all branches and stems, a diminishing frequency toward the tip of the central stem being notable." Whether a variety has a determinate or indeterminate type of development may be correlated with some of the types of morphological response obtained with Morse, Virginia, Lincoln, and T-48. At least on the basis of whether a terminal inflorescence is or is not formed under average field conditions, Morse and Lincoln are indeterminate and Virginia and T-48 are determinate types. The varieties Morse and Lincoln showed highly significant differences in total nodes with variation of nitrogen concentration on long photoperiods. Morse did not initiate terminal inflorescences on any photoperiods. Lincoln likewise failed to form terminal inflorescences, except for a few plants in experiment II in which they were just beginning to form at the time of harvest. On the other hand, Virginia and T-48, which displayed substantially less variation in total nodes with variation in nitrogen concentration on long photoperiods, initiated terminal inflorescences on all photoperiods up to 14 or 16 hours. It is of further interest that Virginia and T-48 are types which can show significantly less variation of yield due to variations in soil fertility than do Morse and Lincoln.

The type of data obtained from these experiments may have some bearing on the choice of varieties for late planting. They suggest that, if it were necessary to plant at such a time that the seedlings would immediately be subjected to short photoperiods, Morse might be more desirable than Virginia. Under such condition Virginia not only would begin to initiate flowers almost as soon as the plants came up but the plant would also form terminal inflorescences, thus limiting the number of places where

flowers and fruits could be borne. On the other hand, Morse plants would be expected to form first-flower buds slightly later than Virginia and to continue the differentiation of nodes and internodes at their terminals for a longer time, with the result that there would be more places for flower and fruit development.

Soybeans planted in the field during the late spring make their early growth at a time when photoperiods are longest and therefore least conducive to flowering. The data of this paper show that it is under these photoperiodic conditions that the plants of certain varieties exhibit the greatest differences in their response to variations in nitrogen treatment. These differences have been measured only in terms of variations in time and place of formation of flower primordia and in certain other morphological characteristics, and it is not known to what extent they may be responsible for ultimate differences in yield. It is significant, however, that rather great differences occur with respect to these plant characteristics in response to nitrogen treatment and that varieties differ rather markedly in the extent to which they exhibit such responses.

Summary

1. In a study of the effects of photoperiod and nitrogen nutrition on the expression of certain morphological characteristics of soybean, plants of Morse, Virginia, Lincoln, and T-48 varieties were grown either in controlled-environment rooms or in the greenhouse and in either soil or sand cultures.

2. In experiments in which nitrogen nutrition was not a factor, all varieties flowered on all durations of photoperiod, but first-flower primordia were formed at a higher node on the plant axis with the longer photoperiods.

3. Position of first-flower primordia did not vary with nitrogen treatment when the plants were grown with short photoperiods. When grown with long photoperiods, however, plants of certain varieties initiated first-flower primordia at higher nodes as the amount of nitrogen in the nutrient solution was increased. Variety T-48 was outstanding in this respect, Lincoln was intermediate, while Morse and Virginia were least responsive.

4. Plants of Lincoln and Morse grown on long photoperiods showed no effect of nitrogen concentration on the number of nodes bearing flower primordia, while there was an effect with Virginia and T-48. The number of flower nodes increased with increasing nitrogen concentration in the case of Virginia, while the opposite trend was true for T-48.

5. The total number of nodes per plant increased with increasing nitrogen concentration when Lincoln, Morse, and Virginia were grown on photoperiods that delay flowering, while this was not true for plants of the T-48 variety.

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THE ORGANIC ACIDS OF LEMON FRUITS[1]

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Introduction

The organic acids are the chief soluble constituents of lemon juice. As the fruit matures, the free acidity of the juice increases and the pH decreases. These changes are determined by estimating the organic acids by titrating a known volume of juice with standard NaOH, with phenolphthalein as indicator, and expressing the result as citric acid. The total acidity, as usually reported for lemon juice, represents the free acids determined in this way. Such values, however, do not show the relation between the kinds and amounts of organic acids in lemon fruits. The present study was carried out to determine these factors. The experiments involved the sampling of fruits of different sizes from a given plot of trees, at intervals during the

[1] Paper no. 534, University of California Citrus Experiment Station, Riverside, California.

growing season, and the subsequent determination, on the juice of each sample, of the soluble solids, the pH, the potentiometric titration curve, the free acids determined on both the juice and the lead acetate precipitate, the citric and malic acids, and the alkalinity of the ash. From these data, the relation of pH to the free and combined acids was determined.

In studies on the organic acids of orange juice, largely from mature fruits, SINCLAIR, BARTHOLOMEW, and RAMSEY (7) have shown that the total acidity of the juice is due chiefly to citric and malic acids, and that the variations in acidity are due chiefly to changes in the citric acid concentration (8.38–25.39 mg./ml. of juice). They found, also, a definite relation between the free-acid–combined-acid balance and the pH of the juice. The concentration of combined acids in the

juice is remarkably uniform; this means that the free-acid concentration is the chief variable.

In other studies on Valencia oranges, SINCLAIR and RAMSEY (8) showed that the maximum amount of free acid was developed in the fruit early in the season and changed very little up to maturity. The concentration of free acids (milligrams per milliliter) in the juice, however, decreased considerably during fruit development. This decrease in free acidity with the corresponding increase in pH value was due chiefly to the decrease in concentration of citric acid brought about by an increase in volume of the fruit during the growing season. Although the malic acid concentration (milligrams per milliliter) in the juice stayed nearly uniform during the season, the actual amount in the fruit increased. The concentration of combined acid remained nearly uniform in the fruit, but the absolute amount per fruit increased. The amounts of combined acid determined from the alkalinity of the ash were in agreement with the values determined from the difference between the total- and free-acid radicals. During ripening, the changes in pH of the juice were definitely related to changes in percentages of the total-acid radical in the free form. A similar relation was noted between pH and the percentage of free acid expressed on a fresh-weight basis.

BARTHOLOMEW (1), in earlier studies, observed the decrease in pH value of lemon juice with the age of the fruit. Juice of Eureka lemons picked monthly between September and July showed a decrease in pH from 4.46 to 2.29. The greatest change in pH (4.46–2.91) occurred during the first 2 months of the sampling period and was accompanied by an increase in the water content of the fruit (53.97–75.42%). Although the ti-

tratable acidities are not recorded for these experiments, the large decrease in pH during the first half of the experimental period would necessitate a corresponding increase in the amount of free acid in the juice.

CALDWELL (2) has shown that, for a short period after the fruit has set, oranges and grapefruit have a pH value close to that found in the vegetative parts of the plant. During this period of low acid and high pH, the fruit has a high total-solids content. Subsequent to this period there is a short transition period (as compared with the total time required for fruit development), in which the pH rapidly decreases from the high values of the vegetative tissues to the low values of the growing fruit. This condition leads to an accelerated rate of water absorption, resulting in a reduction of total-solids content of the fruit and maximum hydration of the tissues.

Material and methods

The juice was extracted from the fruit samples (thirty to eighty lemons each, the number depending upon the size of the fruit), and a 300-ml. aliquot portion was centrifuged and used for the analyses. The refractive index was determined with an Abbé refractometer at 27° C., and the percentage of soluble solids and the specific gravity were read from a sucrose table. All pH values were determined with a Beckman glass-electrode pH meter at 23° C. For the titrations and the citric acid analyses, the juice was diluted with distilled water so that 25 ml. was equivalent to 1 ml. of juice.

The free-acid content of the juice samples was determined by two methods: (a) phenolphthalein titration and (b) potentiometric titration. The inflection point on the (potentiometric) titration curve

was taken to represent most accurately the amount of free acid in the juice.

The quantitative determination of citric acid, free and combined, was made on the juice samples by the pentabromacetone method of PUCHER et al. (6). The sample was heated with H_2S to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by $KMnO_4$ in the presence of KBr. After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S and subsequently titrated with standard $AgNO_3$. The citric acid in the original sample was calculated from the titration. Since this procedure specifies a quantity of citric acid of 1 to 20 mg., it was necessary to modify the method slightly when juice samples containing more than 20 mg. citric acid per milliliter were being studied. The only change necessary was an increase in the amount of standard $AgNO_3$ added in the final steps in order to measure the bromide ion present. With lemon juice containing approximately 5% citric acid, an aliquot portion of diluted juice equivalent to 0.25 ml. pure juice was used for the pentabromacetone analysis.

The malic acid was precipitated from the juice by a procedure described by HARTMANN and HILLIG (5). The organic-acid fraction was precipitated from an alcoholic solution (80%) with lead acetate and the precipitate separated from the filtrate by centrifuging. The precipitate was washed twice with 80% alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulphide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. The malic acid was determined on aliquot portions of this solution by the pentabromacetone method of PUCHER et al. (6). The principle of the method involved the oxidation of malic acid with $KMnO_4$, in the presence of KBr, to a bromine compound volatile with steam. This compound reacts with dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. A pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

The total acids (free and combined) were determined on the clear filtrate by the method of HARTMANN (3). An aliquot portion was evaporated to near dryness (one-tenth original volume), diluted (if necessary) to approximately 30 ml. with distilled water, and titrated with standard NaOH. The results were reported as citric acid. This value is equivalent to the total polybasic acids in the juice.

The amount of combined organic acid in the juice was found experimentally by measuring the alkalinity of the ash from an aliquot portion of juice. For each sampling, four 50-ml. aliquots of juice were ashed at 450° C. to a white residue. To this ash was added 5 ml. of standard 2N HCl; the whole was washed into a 100-ml. volumetric flask and diluted to volume with water; a 10-ml. aliquot was then titrated with standard NaOH. The difference in titer of NaOH between the sample and a blank solution of the HCl represents the alkalinity of the ash. The milliequivalents of HCl neutralized by the ash closely approximate the milliequivalents of combined organic acid in the juice, since these organic salts decompose to carbonates during ashing.

A plot of thirty lemon trees (fifteen Eureka and fifteen Lisbon) was the source of the fruit used in studying the relation of fruit size to the pH and free acidity of the juice. All fruits used in this

particular experiment were tagged and measured when 1.5–2.0 cm. in diameter. As lemon trees bloom and set fruit in each month of the year, it is necessary to know the size of the fruit at the beginning of the experiment in order to correlate the size with the age of the fruit. To secure the juice from small fruits 2–3 cm. in diameter, they were peeled carefully with a knife. The resulting pulp, which amounted to one-fourth to one-third the

TABLE 1

RECOVERY OF MALIC ACID FROM PURE SOLUTIONS

| MALIC ACID TAKEN (MG.) | MALIC ACID RECOVERED BY | | | |
| | Gravimetric method | | Colorimetric method | |
	mg.	%	mg.	%
1.00	0.99	99.0	1.00	100.0
1.00	1.06	106.0	0.95	95.0
1.00	0.99	99.0	1.03	103.0
2.00	1.99	99.5	2.08	104.0
2.00	2.06	103.0	2.03	101.5
2.00	1.99	99.5	2.00	100.0
3.00	3.06	102.0	2.98	99.3
3.00	2.99	99.6	2.98	99.3
3.00	2.92	97.3	2.90	96.6
5.00	5.06	101.2	5.20	104.0
10.00	10.25	102.5	9.90	99.0
Mean		100.8		100.2

volume of the whole fruit, was placed in a jar and frozen solid in a cold-storage chamber. After thawing, the pulp was subjected to a pressure of 20,000 pounds per square inch in a hydraulic press. The expressed liquid was centrifuged and analyzed.

Results

DETERMINATION OF MALIC ACID IN LEMON JUICE

The malic acid values determined by the colorimetric method represent to a considerable degree of accuracy (within 5%) the actual amount of malic acid in

the juice samples. Nevertheless, it was thought desirable to compare the gravimetric and colorimetric methods on pure solutions of malic acid. The results obtained by both methods (table 1) gave satisfactory agreement.

In the gravimetric procedure, the factor reported by HARTMANN (4) was used to convert the hydrazine precipitate into malic acid (1 gm. hydrazine precipitate = 0.712 gm. malic acid). This factor is not significantly different from the experimental value determined in the present studies (1 gm. hydrazine precipitate =

TABLE 2

RECOVERY OF MALIC ACID ADDED IN VARIOUS AMOUNTS TO LEMON JUICE*

| EXPERI- MENT NO. | MALIC ACID CONTENT (MG./ML.) | MALIC ACID ADDED (MG./ML.) | MALIC ACID RECOVERY FROM LEAD-ACETATE PRECIPITATE | | |
			Total (mg./ml.)	Added (mg./ml.)	%
1	2.30	0.00	2.30	0.00	
2	2.30	0.50	2.80	0.50	100.00
3	2.30	1.00	3.36	1.06	106.00
4	2.30	1.50	3.82	1.52	101.33
5	2.30	2.00	4.40	2.10	105.00

* Juice sample contained 63.90 mg./ml. citric acid.

0.715 gm. malic acid). The precipitates that were weighed in the gravimetric determinations were obtained by distilling the volatile bromine compound of malic acid into dinitrophenylhydrazine and were not the result of direct precipitation from the oxidized solution (4).

The thoroughness with which lead acetate precipitates malic acid from lemon juice is indicated by the percentages of recovery listed in table 2. Different amounts of malic acid were added to aliquot portions of a lemon-juice sample and subsequently precipitated with lead acetate in 80% alcohol. The total amount of malic acid in this precipitate was determined as previously described. The lemon juice used for these determinations contained 63.90 mg. citric acid and 2.30

mg. malic acid per milliliter. The high concentration of citric acid served to test the effect of this organic acid on the recovery of malic acid.

RELATION OF FREE-ACID FRACTION TO CITRIC ACID CONTENT OF LEMON JUICE

In previous studies on orange juice (7, 8), the free acid calculated from the volume of standard NaOH required for neutralization, with phenolphthalein as indicator, was higher than the amounts determined by potentiometric titration.

lemons ranging from 4.0 to 7.0 cm. in diameter. The inflection point on the titration curve of lemon juice occurred at approximately pH 8.10, in the region where phenolphthalein gave an initial color change. In this region the curve of lemon juice, like that of pure citric acid, is perpendicular to the abscissa, and only a very small increment of NaOH is needed to produce a large change in pH.

The actual amount of citric acid in the lemon-juice samples, as determined by the pentabromacetone method (6), is shown

TABLE 3

RELATION OF FREE-ACID FRACTION TO CITRIC ACID CONTENT OF LEMON JUICE

SAMPLE NO. AND VARIETY	SAMPLING DATE	STAGE OF FRUIT MATURITY	FRUIT DI-AMETER (CM.)	TOTAL SOL-UBLE SOL-IDS (%)	pH	FREE ACID (AS CITRIC) DETERMINED BY				CITRIC ACID (PENTABROM-ACETONE METHOD)		COMBINED ACIDS (AS CITRIC) DE-TERMINED FROM ALKA-LINITY OF ASH	
						Phenolphthal-ein titration		Potentiometric titration					
						mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.
1. Lisbon	11/30/44	Green	5.0	9.19	2.30	54.54	0.852	53.74	0.840	54.32	0.849	2.21	0.035
2. Eureka	12/6/44	Green	4.5	8.31	2.30	48.15	0.752	48.15	0.752	47.88	0.748	2.44	0.038
3. Eureka	12/12/44	Green	4.5	9.35	2.25	58.09	0.923	58.80	0.919	53.08	0.829	3.10	0.049
4. Lisbon	12/17/44	Silver	4.5	9.48	2.26	61.50	0.961	61.50	0.961	64.06	1.015	2.90	0.045
5. Lisbon	12/26/44	Silver	5.7	9.00	2.38	58.07	0.907	57.88	0.904	52.00	0.812	2.90	0.045
6. Eureka	1/10/45	Yellow	7.0	7.96	2.22	53.01	0.828	53.01	0.828	54.47	0.851	2.03	0.032
7. Eureka	1/16/45	Green	5.0	8.30	2.20	51.83	0.810	51.83	0.810	48.83	0.763	2.06	0.032
8. Lisbon	1/18/45	Yellow	5.0	9.01	2.18	56.68	0.885	56.68	0.885	56.45	0.882	1.60	0.025
9. Lisbon	1/22/45	Yellow	6.5	9.17	2.15	58.47	0.913	58.80	0.919	59.14	0.924	1.80	0.028
10. Lisbon	1/27/45	Yellow	5.5	8.54	2.22	55.15	0.862	55.48	0.867	54.10	0.845	2.06	0.032

This was because the phenolphthalein changes color between pH 8 and 9, and more NaOH is required to reach the end point with phenolphthalein than to titrate the corresponding sample potentiometrically to pH 7.80—the inflection point on the titration curve. The titration curve of pure citric acid is nearly perpendicular to the abscissa in that portion of the curve close to the neutral point, but the titration curve of orange juice has considerable slope within a similar region of the curve.

With lemon juice, however, the free-acid values determined by the phenolphthalein and potentiometric titration methods gave good agreement (table 3). This was true for both green and mature

in table 3. These values include both the free citric acid and that combined in the form of citrates. The total citric acid content is approximately equal to the free acid. It can be seen that additional organic-acid radicals are needed to account for the salt formation in the juice. These are supplied by the malic acid in the juice, as reported in table 5. The amount of organic acid in the salt form is reported as combined acid (table 3), calculated as citric acid from the alkalinity of the ash. The concentration of combined acid is comparatively uniform, when the wide range of maturity of the fruit samples is considered.

The citric acid content of various lemon-juice samples, determined on the

pure juice and on the lead-acetate precipitate, is recorded in table 4. Attention should be drawn to the close agreement between the citric acid values determined in the two cases. Apparently, lead acetate gave a complete precipitation of the citric acid radical (free and combined) in the juice. The free acid (as citric) is not significantly different from the actual citric acid content of the pure juice. These data show further that the non-acid constituents in lemon juice do not

TABLE 4
CITRIC ACID VALUES DETERMINED ON PURE LEMON JUICE AND ON LEAD-ACETATE PRECIPITATE

SAMPLE	FREE ACID (AS CITRIC) (MG./ML.)	CITRIC ACID DETERMINED ON	
		Pure juice (mg./ml.)	Lead-acetate precipitate (mg./ml.)
1.........	61.24	62.50	62.27
2.........	55.68	55.21	55.12
3.........	58.80	53.08	53.00
4.........	63.67	62.93	63.02
5.........	70.08	68.99	68.99
6.........	57.85	58.24	58.46
7.........	61.50	64.98	64.53
8.........	58.82	59.14	59.03
9.........	55.25	55.87	56.00
10.........	62.37	65.00	65.00

influence the determination of citric acid by the pentabromacetone method.

POLYBASIC ACIDS OF LEMON JUICE PRECIPITATED WITH LEAD ACETATE

According to HARTMANN (4), the organic polybasic acids occurring in fruits are soluble in alcohol and are precipitated quantitatively as the lead salts from 80% alcohol. Since the acidity of lemon juice is due chiefly to the polybasic acids, it follows that this procedure should serve to isolate them in nearly pure form from the other constituents in the juice. On this basis, the polybasic acids of lemon juice have been isolated

and subsequently determined quantitatively by the oxidation methods. In table 5 the values are recorded as (1) total acid (as citric) titrated with standard NaOH, with phenolphthalein as indicator, (2) citric acid, (3) malic acid, and (4) total citric and malic acids. The first three values were experimentally determined.

The total-acid values (as citric) represent all the acid groups precipitated from the juice with lead acetate. This precipitate included, if present, the lead salts of the fruit acids, the combined acids, and the acid salts of the juice. These titration values are highly accurate, provided the lead-acetate precipitate is thoroughly washed with 80% alcohol to free it of excess lead acetate and acetic acid.

The polybasic acids precipitated from lemon juice with lead acetate are represented by the citric and malic acid values (table 5). It is obvious that the main portion of the total acids of lemon juice is citric acid. Although the concentration of malic acid is small in comparison with that of citric acid, the amount present has to be considered more than a trace, as usually reported in the literature. The malic acid concentration of lemon juice varied widely with the maturity of the fruit, however, the amount decreasing as the fruit ripened. The total-acid constituents (titratable acidity) of the lead-acetate precipitate are equal (within experimental error) to the sum of the citric and malic acids, expressed in milliequivalents per milliliter. This is important because it demonstrates that the actual amounts of citric and malic acids account for all the acid groups precipitated as the lead salts.

If isocitric acid is present in more than microconcentrations, it should appear in the lead-acetate precipitate. Isocitric acid does not yield pentabromacetone (4)

TABLE 5

ANALYSIS OF ORGANIC ACIDS OF LEMON JUICE

SAMPLE NO. AND VARIETY	SAMPLING DATE	STAGE OF FRUIT MATURITY	FRUIT DIAMETER (CM.)	TRUE* SOLUBLE SOLIDS (%)	pH	FREE ACID (AS CITRIC) DETERMINED BY PHENOLPHTHALEIN TITRATION		ACID CONSTITUENTS PRECIPITATED BY LEAD ACETATE								COMBINED ACID (AS CITRIC) DETERMINED FROM ALKALINITY OF ASH		FREE ACID (%)
								Total acid titrated (as citric)		Citric acid (pentabromacetone method)		Malic acid		Total acid (citric+malic)				
						mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	
								Fresh fruit from experimental plots										
1. Lisbon	April 3	Yellow	6.0	8.8	2.20	55.25	0.863	59.02	0.922	56.00	0.875	3.65	0.055	59.54	0.930	1.76	0.028	96.00
2. Lisbon	April 9	Yellow	6.0	8.8	2.15	62.37	0.974	67.52	1.055	65.00	1.015	2.60	0.039	67.48	1.054	2.18	0.034	96.63
3. Lisbon	April 16	Green	4.0	9.7	2.13	55.94	0.874	61.72	0.964	58.52	0.914	2.60	0.039	61.01	0.953	1.88	0.030	96.74
4. Eureka	April 10	Yellow	5.0	9.0	2.25	55.94	0.874	61.21	0.956	59.77	0.934	1.39	0.022	61.20	0.956	1.94	0.030	96.61
5. Eureka	June 8	Green	4.0	8.0	2.33	45.56	0.712	49.59	0.776	46.59	0.728	2.56	0.044	49.42	0.772	1.83	0.029	96.15
6. Lisbon	June 12	Green	4.0	8.7	2.28	53.78	0.840	51.58	0.809	53.33	0.833	4.20	0.065	57.46	0.806	1.90	0.029	96.50
7. Eureka	June 18	Green	4.0	8.3	2.31	45.87	0.716	48.72	0.761	45.33	0.708	3.51	0.052	48.66	0.760	1.79	0.028	96.25
8. Lisbon	June 25	Green	4.0	8.8	2.28	48.09	0.751	53.12	0.830	47.87	0.748	4.28	0.064	51.98	0.812	1.86	0.029	96.27
								Mature fruit from packing-house storage†										
9. Eureka	April 23	Yellow	5.5	8.9	2.25	65.37	0.974	69.05	1.079	67.65	1.057	1.00	0.029	69.53	1.086	2.13	0.033	96.69
10. Eureka	April 25	Yellow	5.5	9.5	2.25	65.37	0.974	65.39	1.021	63.50	0.992	1.80	0.027	65.24	1.010	2.03	0.033	96.76
11. Eureka	April 30	Yellow	5.5	9.6	2.22	67.16	1.049	71.59	1.117	69.80	1.092	2.10	0.032	71.96	1.124	2.13	0.033	96.92
12. Eureka	May 2	Yellow	6.0	7.0	2.29	54.66	0.854	59.16	0.924	57.12	0.892	2.30	0.035	59.34	0.927	1.94	0.030	96.59
13. Eureka	May 7	Yellow	6.0	8.0	2.32	61.50	0.961	65.86	1.028	63.86	0.998	2.30	0.033	66.00	0.031	2.15	0.034	96.62
14. Eureka	May 10	Yellow	4.5	9.0	2.20	61.25	0.957	65.95	1.032	63.95	0.998	2.30	0.035	66.15	1.033	1.90	0.030	97.00
15. Eureka	May 14	Yellow	5.5	10.3	2.11	70.16	1.095	76.05	1.188	74.28	1.160	2.35	0.034	76.44	1.194	2.08	0.033	97.11

* Owing to the relatively high acid content of the juice, a correction was added to the refractometer sucrose values to give the true percentage of soluble solids (g).

† Samples taken from different lots of fruit that had been in storage from 1 week to 3 months.

and consequently has no influence on the citric acid values determined by the penta-bromacetone method. The only possibility left would be for the isocitric acid to form another compound during the oxidation process and cause a slight increase in the malic acid values.

RELATION OF pH TO FREE AND COMBINED ACIDS IN LEMON JUICE

The free-acid fraction of lemon juice is due mostly to citric acid. The combined-acid fraction is calculated as citric. The error in this calculation is small, since the malic acid content is very low in comparison with the citric acid content, and the equivalent weights of the two acids are nearly equal (citric acid 64.02, malic acid 67.02). It is seen then that the buffering action of lemon juice is due to the weak acid (citric) and a salt, or salts (citrates), with a common anion. The pH of the juice is therefore determined chiefly by the dissociation constant (pK_a) and the ratio of combined acid to free acid. Other constituents that may contribute to the hydrogen-ion concentration in lemon juice are in such small amounts, in comparison with the citric and malic acid content, as to have little effect.

The relation of pH to the concentration of free acid (as citric, in milligrams per milliliter) in different samples of lemon juice is shown in table 5. The juice from fruits that ranged from 4.0 to 6.0 cm. in diameter showed only a slight variation in pH. Although the free acid (as citric) varied from 45.56 to 70.10 mg./ml., the percentage of total acid in the free state showed little variation. This means that the combined acids (salts of inorganic cations) did not vary enough to produce more than a slight shift in pH. The average amount of citric acid in the combined form was 1.97 mg./ml. This relation is further borne out by the titra-

tion curve of lemon juice shown in figure 1. These small fluctuations of pH correspond to changes in the salt content of 3.00–4.00%.

The relation of pH to the percentage of total acid in the combined form (salt) is well illustrated in figure 1 by the titration curves of lemon juice and of pure citric acid solutions. The concentration of the citric acid solution used in securing the data for one of the curves was 62.00 mg. citric acid per milliliter; the concentration of free acid in the lemon-juice sample was equivalent to 61.50 mg. citric acid per milliliter. Both the citric acid solution and the lemon-juice sample had, intentionally, nearly the same concentration of free acid. The initial pH value of this pure citric acid solution was 1.82; that of the lemon juice was 2.32. The initial point on the lemon-juice curve (fig. 1) was corrected for the salt occurring naturally in the juice (3.40% at pH 2.32). The percentage of acid neutralized, for all points other than the initial one, included the combined acids present in the original sample and the sodium citrate formed by the neutralization of part of the free acid. For purposes of this study, curves representing only 50% of the total acid neutralized have been plotted on the graph.

The slight difference between the curves (fig. 1) is indicated by the fact that the pH values on the lemon-juice curve are slightly higher than those on the citric acid curves, for a given amount of combined acid (salt). The curves are nearly parallel and consequently have nearly the same slope. The close similarity of these curves is evidence that lemon juice titrates like a pure citric acid solution, because the free acid in the juice is composed chiefly of citric acid, and the ratio of free acid/combined acid is sufficiently great to diminish the salt effect

on the pH. The quantity of cations available in the juice for combining with the organic acids is relatively small in comparison with the concentration of free acids. Nevertheless, to designate the pH-salt relationships accurately, a correction must be made for the combined acid naturally occurring in the juice. In titrating lemon juice with a strong base, it is possible to calculate the pH at any salt/acid is unity, and the pH is equal to pK_a. At this point also the titration curve has a minimum slope and, consequently, a maximum buffer efficiency.

In previous papers on orange juice (7, 8) it was shown that the combined acids calculated as the difference between the free acid and the sum of the citric and malic acids were approximately the same as the combined acids determined
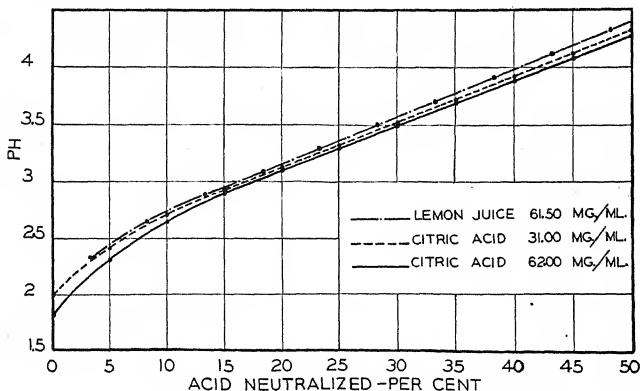
FIG. 1.—Titration curves of lemon juice and of pure citric acid solutions, showing relation of pH to salt content, up to 50% of acid neutralized. Initial point on lemon-juice curve was corrected for salt occurring naturally in the juice.

point on the curve preceding the end point, with the familiar equation pH = pK_a + log (salt/acid).

Since lemon juice titrates like a pure acid solution, its buffer value is equal to that of any other weak acid. All weak acids have dissociation curves of the same shape and have equal buffer capacities at any given degree of dissociation (or fraction of acid neutralized). Lemon juice, like any weak acid, has the greatest buffer efficiency when 50% of its acid is in the salt form. At this point the ratio

from the alkalinity of the ash (all values calculated in milliequivalents). With lemon juice, however, the difference between the free-acid fraction and the total organic-acid radical (citric and malic) is relatively much greater than the combined acids determined from the alkalinity of the ash (table 5). Experimentally this means that a small portion of the acid radical was in a form other than free acid or salt of the inorganic cations, but that it was accounted for in the citric and malic acid fractions of the juice.

Since this particular form of the combined-acid radical was not titrated with NaOH, or did not exist in the juice as a salt of inorganic cations, the pH and titration curve were not affected on the acid side of the inflection point. With respect to factors that affected the pH, the alkalinity of the ash gave a true combined-acid content of the juice. This is well demonstrated in figure 1. The difference in the combined acids determined by the two methods strongly indicated

large increase in free acid. Further increase in fruit size, up to a diameter of 6.0 cm., resulted in a decrease of approximately 0.3 of a pH. After the free acid reached a certain concentration, which apparently appears when the fruit is about 4.0 cm. in diameter, the pH changed very little. The free acids, on the other hand, continued to increase gradually after the fruits had reached this diameter.

It should be emphasized that these
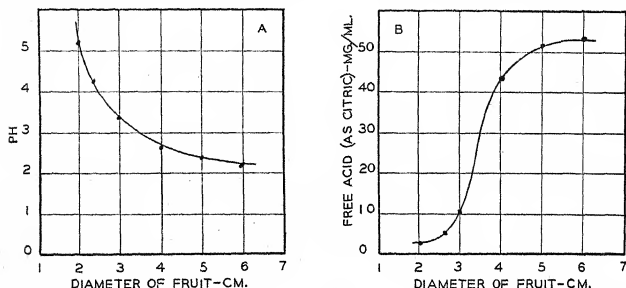
Fig. 2.—Changes in pH and in free acid with fruit growth. Each point on the curves represents the mean of many determinations. Fruits sampled had been previously tagged when 1.5–2.0 cm. in diameter.

that a small portion of the citric or malic acids existed in the ester form. This compound in the juice would fit the conditions just cited.

CHANGES IN pH AND FREE ACID OF LEMON JUICE WITH FRUIT GROWTH

The pH and free-acid values of the juice of lemon fruits of different sizes are shown in figure 2. Results show the increase in free acids and decrease in pH with increase in fruit size. As the equatorial diameter of the fruit increased from 2.0 to 4.0 cm., the pH value of the juice decreased from 5.20 to 2.60. The large reduction in pH at this period of fruit growth is due to a correspondingly

fruits were picked according to size from the same trees throughout the sampling period. Each value on the two curves, A and B (fig. 2), is the mean of many determinations on fruits of the same bloom. These curves are trends and represent the change in pH and free acid in the juice of fruits of known ages. As lemon trees bloom and set fruit in each month of the year, the relationships shown in figure 2 would probably not hold for fruits picked at random at any one time. This is borne out by the data of table 5, which show that the juice of fruits 5.5 cm. in diameter may have more free acid (67.19 mg./ml.) than that of fruits 6.0 cm. in diameter (54.66 mg./ml.). To

overcome most of this difficulty, the fruits that were sampled had been previously tagged when they were 1.5–2.0 cm. in diameter. The size of the fruit may not indicate its age, unless fruits of the same bloom are used. BARTHOLOMEW (1), by tagging a great number of fruits and measuring at monthly intervals, showed the relation between the age and the diameters of lemon fruits. The curve (fig. 2) showing the change in pH with the size of fruit is in good agreement with BARTHOLOMEW's data showing the

FIG. 3.—Changes in pH with variations in concentration of free acid in lemon juice during fruit development.

change in pH of lemon juice with the age of the fruit. It is evident, therefore, that if the pH and total acids, respectively, were plotted against the age of the fruit rather than against the size, the trend of the curves would be the same.

Figure 3 designates the changes in pH with variations in concentration of free acid (milligrams per milliliter) in fruits ranging between 1.5 and 6.0 cm. in diameter. This curve is interesting because it shows the rapid increase in the hydrogen-ion concentration of the fruit, from the low values approaching those of the vegetative tissues to the high values of the growing fruit. This curve demonstrates again that when the free acid reached a concentration of about 40 mg./ml., fur-

ther increases produced only slight changes in pH.

Summary

1. The acidity of lemon juice is due chiefly to citric and malic acids. If other acids are present, they exist in very small amounts. The citric acid determined on the pure juice does not differ significantly from the amount precipitated by lead acetate. The total acid content precipitated from lemon juice by lead acetate is equal (within experimental error) to the sum of the citric and malic acids.

2. The difference between the free-acid fraction and the total organic-acid radical (citric and malic) is relatively much greater than the combined acids determined from the alkalinity of the ash. Since this difference is not reflected in the titratable acidity or pH values, it probably represents the quantity of organic acid in the ester form. The alkalinity of the ash represents the organic acid combined in salt form with the excess inorganic cations.

3. The titration curve of lemon juice is very similar to that of a pure citric acid solution, provided a correction is made for the combined acid naturally occurring in the juice. There is a definite relation between the pH and the amount of acid in the salt or combined form. The small fluctuations in pH of mature lemon juice are correlated with the large ratio of free acid/combined acid.

4. The free acids (milligrams per milliliter) increased and the pH of the juice decreased with increase in fruit size. The large reduction in pH (5.20 to 2.60) which occurred in fruits 2.0–4.0 cm. in diameter was due to a correspondingly large increase in free acid. Further increase in fruit size (up to 6.0 cm. in diameter) resulted in a slight decrease of approximately 0.3 of a pH, while the free

acids gradually continued to increase in the juice.

For the past 4 years, investigations on the organic acids of citrus fruits have been in progress at this station. During this period we have had at all times the benefit of discussions with the following members of the Research Department of the California Fruit Growers Exchange: Messrs. W. E. BAIER, J. W. STEVENS, and P. W. ROHRBAUGH; and Drs. GLENN H. JOSEPH and EDWIN F. BRYANT.

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CARPELLARY AND PLACENTAL STRUCTURE
IN THE SOLANACEAE

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Introduction

There has been much speculation on the exact morphology of the carpel and placentae in the Solanaceae. The axile placentae and cauline ovules ascribed to this family by most workers are difficult to reconcile with the Candollean theory that all floral parts are specialized leaves. According to the classic theory, an ovary is composed of one or more carpels, each of which is morphologically comparable to a folded leaf bearing ovules on its margins. In a syncarpous ovary, however, the carpels are undiverged, and the axis may extend into the gynecium. The problem is to determine whether the carpel walls, the septa, and the ovule-bearing portions of the placentae are cauline, foliar, or both.

A total of fourteen genera and twenty-one species of this family were selected for investigation, including members with fleshy and with only slightly fleshy placentae. On the basis of the anatomy of each flower, it was noted that WETTSTEIN's (23) phylogenetic arrangement of the genera agrees rather closely with a sequence based on floral anatomy. While this paper is not specifically one on phylogeny, the phylogenetic approach proved the best method of co-ordinating the material.

The Solanaceae contain many plants of economic importance. These can be divided into three groups. *Solanum tuberosum* (potato), *S. nigrum* var. *guineense* (garden huckleberry), *S. melongena* var. *esculentum* (eggplant), and species of *Lycopersicon* (tomato) and *Capsicum* (pepper) are sources of food. *Nicandra physalodes* and species of *Lycium*, *Datura*, *Nicotiana*, *Petunia*, *Nierembergia*, *Salpiglossis*, *Schizanthus*, *Cestrum*, *Brunfelsia*, *Solanum*, and *Browallia* are cultivated as ornamentals. Several members are grown for the alkaloids they contain. These include *Atropa belladonna*, *Hyoscyamus niger*, *Datura stramonium*, and species of *Nicotiana*.

The flowers of the Solanaceae are hypogynous and generally actinomorphic, calyx four- to six-lobed and persistent, corolla sympetalous and generally five-lobed, stamens five, and the filaments adnate to the corolla and alternate with the corolla lobes. The ovary is usually bicarpellate, but in some plants there may be three to five carpels. The ovules are generally numerous and borne on more or less fleshy placentae. The fruit is a capsule or a berry. The Solanaceae are most closely related to the Scrophulariaceae but are separated from this family by having bicollateral bundles and an ovary which is oblique in relation to the median plane of the flower.

Among families in which the ovary is superior and bicarpellate and the corolla sympetalous, WERNHAM (22) lists the following tendencies: (*a*) actinomorphy to zygomorphy; (*b*) reduction in number of fertile stamens; and (*c*) reduction in number of ovules per carpel. The first two tendencies are particularly evident in the Solanaceae.

Material and methods

The plants listed were studied. The genera have been arranged according to

the classification of WETTSTEIN (23) in ENGLER and PRANTL's *Die Natürlichen Pflanzenfamilien:*

er flowers are actinomorphic, the stamens are fertile and of equal size, and there are two adaxial bundles to each

Nicandreae . . . .   *Nicandra physalodes* Pers.

Solaneae-Lyciineae . . . . { *Lycium halimifolium* Mill.
                                       { *Atropa belladonna* L.

Solaneae-Hyoscyaminae . . . . .   *Hyoscyamus niger* L.

Solaneae-Solaninae . . . . { *Physalis alkekengi* L. (*P. francheti* Hort.)
                                       { *Capsicum frutescens* L. var. *grossum* Bailey
                                       {   (Early Wonder)
                                       { *Solanum tuberosum* L.
                                       { *S. nigrum* var. *guineense* L.
                                       { *S. melongena* L. var. *esculentum* Nees.
                                       { *S. pseudocapsicum* L.
                                       { *S. dulcamara* L.
                                       { *S. rostratum* Dunal
                                       { *Lycopersicon pimpinellifolium* Mill. (included
                                       {   under *Solanum* by WETTSTEIN)

Datureae . . . . { *Datura stramonium* L.
                                       { *D. meteloides* Dunal

Cestreae-Nicotianae . . . . { *Nicotiana sanderae* Sander (Crimson Bedder)
                                       { *N. glauca* Graham
                                       { *Petunia hybrida* Vilm. (Snowstorm-Giant Single)
                                       { *Nierembergia hippomanica* Miers

Salpiglossideae . . . . { *Salpiglossis sinuata* Ruiz and Pav.
                                       { *Schizanthus pinnatus* Ruiz and Pav.

The flowers and fruits were collected at various stages in development, killed and fixed either in Navashin's solution or in formalin-acetic-alcohol, dehydrated by the usual tertiary butyl-alcohol method, and imbedded in paraffin. Both longitudinal and transverse serial sections were cut at 12 μ, although some of the large fruits were sectioned at 20 μ. The material was then stained in a modified Flemming's triple stain.

Observations

Of the flowers studied, those of Nicandreae, Solaneae, and Datureae follow essentially the same pattern of floral anatomy. The exceptions are *Solanum rostratum*, which appears to be an anomalous form, and *Hyoscyamus niger*, which has slightly irregular flowers. All the oth-

carpel. *Physalis alkekengi* has been chosen as typical of this group.

The pedicel of *P. alkekengi* is an amphiphloic siphonostele; in the receptacle the vascular tissue consists of ten distinct bundles (fig. 25). The first five divergences extend outward and are the median sepal bundles. At the periphery, lateral divergences from the sepal bundles are anastomosed with one another (fig. 26). Slightly distal to the main sepal divergences, the vascular cylinder is again continuous. Next there are five petal divergences (fig. 26) alternating with the sepal bundles and accompanied by small parenchymatous gaps. At each side of the main petal bundle there is a lateral divergence, so that the corolla has fifteen prominent bundles in all. The vascular tissue is again continuous at a

level slightly distal to the petal divergences. Opposite the median sepal bundles and alternate with those of the petals, five stamen bundles diverge from the stele without accompanying gaps (fig. 27). After the divergence of the first three sets of floral parts, the vascular cylinder is elliptical and again continuous. In the middle of the long arcs of the ellipse two large bundles (the adaxials) diverge toward the center of the ovary and are accompanied by gaps. At almost the same level the remaining portion of the ellipse is broken into small bundles which diverge outward slightly and then upward. These are bundles of the carpellary walls. The abaxial bundles of the carpels begin at the middle of the two short arcs of the ellipse, diverge outward at a slightly more distal level than the other carpellary bundles, and extend upward the length of the style (figs. 27, 28).

There are usually two carpels in *Physalis*. The abaxial bundle of one carpel is opposite the mid-bundle of a petal, while the other abaxial bundle is opposite a stamen bundle (fig. 29). At the level at which the two adaxial bundles are almost in the center of the ovary, four cavities become evident between the adaxial bundles and those of the carpellary walls. Then each adaxial bundle is divided into two, one for each carpel. The tissue between the two cavities in each carpel or the tissue between the carpel wall and the placenta extends for only a short distance. Distal to this, the ovary consists of the carpel walls, the two loculi, and two massive placentae bearing ovules which are supplied with vascular tissue through divergences from the adaxial bundles (fig. 29). At the top of the ovary the two septa do not meet completely, so that the uppermost portion of the ovary and the base of the style appear to be unilocular. Still farther up the style there is no cavity, the entire central portion being occupied by transmitting tissue. The smaller anastomosing bundles of the carpellary walls and the adaxial bundles are not present in the style, but the abaxial bundles extend to the stigma (fig. 30).

From the standpoint of interpretive morphology it is necessary to note the position of the xylem and the phloem in the adaxial bundles. These bundles diverge from an amphiphloic siphonostele and remain bicollateral until they are divided at the level where the four cavities first become evident. Then the xylem is separated into two groups by parenchymatous rays, phloem is differentiated completely around each group of xylem, and the bundles are amphicribral.

The other genera of Nicandreae, Solaneae, and Datureae follow rather closely the floral anatomy of *Physalis*. There are, however, some differences. In *Lycium halimifolium* the five divergences to the sepals may or may not be accompanied by very small gaps. After these first divergences, the stele is star-shaped. From each of the five points there is a divergence—the lateral sepal bundle. Smaller lateral bundles may be formed either through trifurcation of the main lateral or as divergences from this bundle after it is already differentiated (fig. 14). *Hyoscyamus niger* differs from all other Solanaceae studied in the formation of its sepal bundles. The divergences to the sepals are not separate traces but an entire ring of tissue. Certain portions of this outer cylinder stain darker and become differentiated into five large median, five large lateral, and an indefinite number of small anastomosing bundles. The ring of darker-staining tissue remains in the calyx and becomes lignified in the mature flower (figs. 19–23). *Capsicum frutescens* var. *grossum* and *Solanum melongena* var.
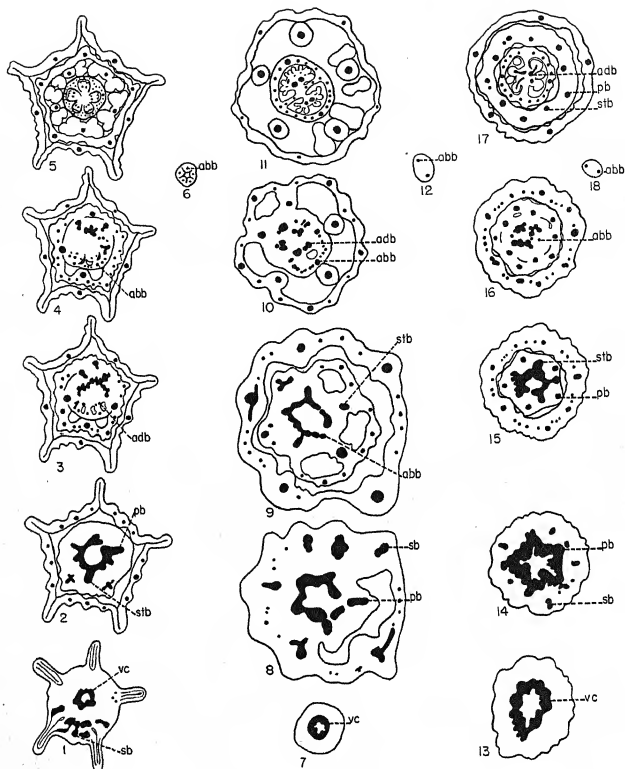
FIGS. 1–18.—Transections of flowers from pedicel to apex showing comparable stages in floral development. Species in figs. 1–90 arranged in phylogenetic sequence. Figs. 1–6, *Nicandra physalodes*. Figs. 7–12, *Atropa belladonna*. Figs. 13–18, *Lycium halimifolium*. Figs. 10, 11, shown without sepals. Figs. 6, 12, 18, style. (*vc*, vascular cylinder; *sb*, sepal bundle; *pb*, petal bundle; *stb*, stamen bundle; *adb*, adaxial bundle; *abb*, abaxial bundle.)
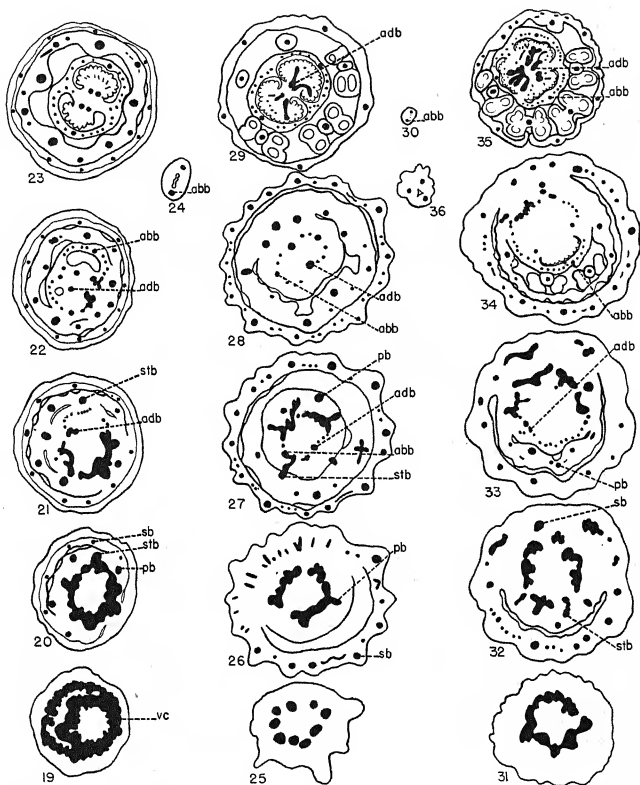
FIGS. 19–36.—Transections of flowers from pedicel to apex. Figs. 19–24, *Hyoscyamus niger*. Figs. 25–30, *Physalis alkekengi*. Figs. 31–36, *Capsicum frutescens* var. *grossum*. Figs. 29, 35, shown without sepals. Figs. 24, 30, 36, style.
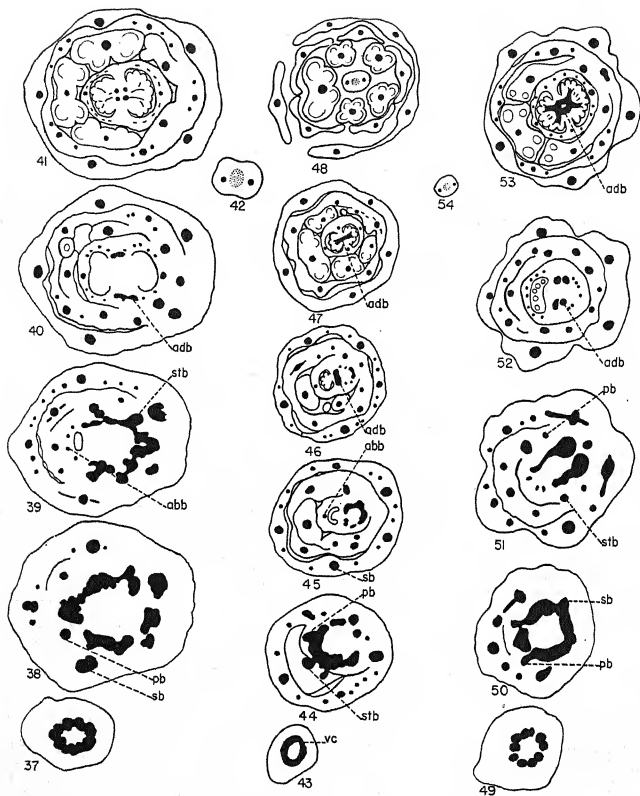
Figs. 37–54.—Transections of flowers from pedicel to apex. Figs. 37–42, *Solanum tuberosum*. Figs. 43–48, *Solanum rostratum*. Figs. 49–54, *Lycopersicon pimpinellifolium*. Figs. 42, 54, style.
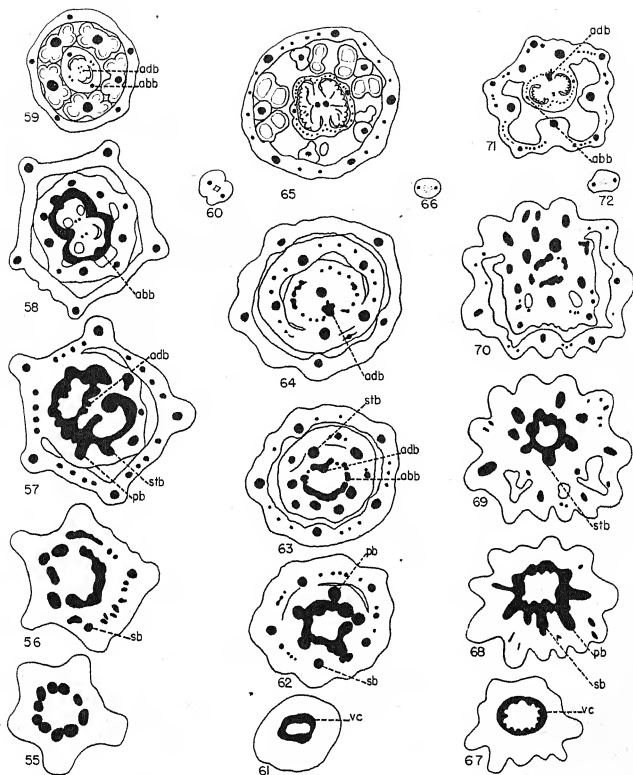
FIGS. 55–72.—Transections of flowers from pedicel to apex. Figs. 55–60, *Datura stramonium*. Figs. 61–66, *Nicotiana glauca*. Figs. 67–72, *Petunia hybrida*. Figs. 59, 65, 71, shown without sepals. Figs. 60, 66, 72, style.
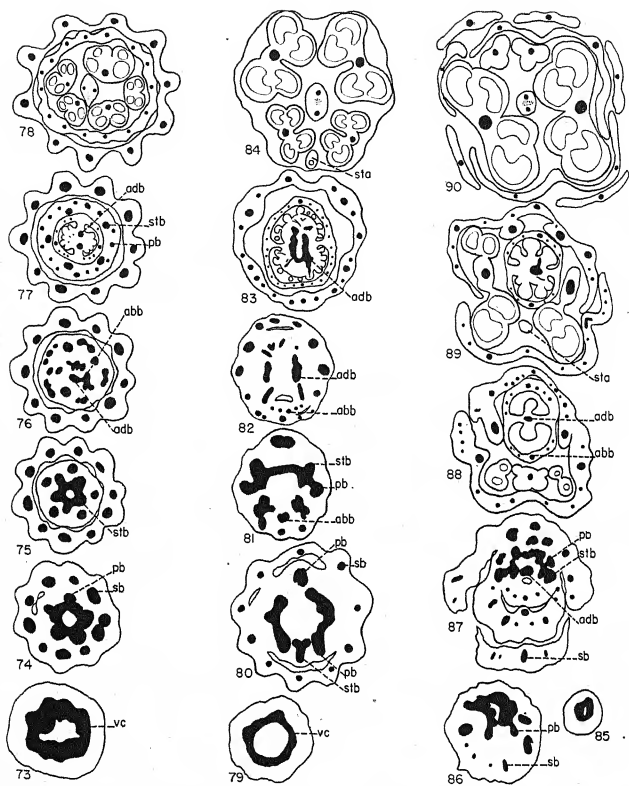
FIGS. 73-90.—Transections of flowers from pedicel to apex. Figs. 73-78, *Nierembergia hippomanica*. Figs. 79-84, *Salpiglossis sinuata;* figs. 81-83, shown without sepals; fig. 84, showing only stamens and style. Figs. 85-90, *Schizanthus pinnatus;* figs. 88, 89, shown without sepals. (*sta*, staminodium.)

esculentum are commercial forms. Because cultivation has brought about a multiplication of floral parts, the floral anatomy of these plants differs somewhat from that of the ancestral types. Pepper has six sepals (figs. 32–34), while eggplant is even more variable. In most of the latter there are eight sepal divergences which do not come from the cylinder at the same level. These divergences are in two sets of four each, those of one set alternate with those of the other and at a level slightly distal to the first four. The anastomoses of the sepal bundles in *Datura stramonium* (fig. 56) and *D. meteloides* form an almost continuous ring of tissue between the main sepal bundles.

The differences in petal and stamen divergences require only brief mention. In *Lycium* (fig. 15), *Capsicum* (fig. 32), *Solanum dulcamara*, and *Lycopersicon* (fig. 50) the petal divergences are accompanied by parenchymatous gaps in the vascular cylinder, but no gaps are associated with the stamen divergences in any plant studied. The vascular cylinder does not again become continuous after the petal divergences in *Capsicum* (figs. 32–34). In the cultivated forms of *Solanum melongena* var. *esculentum* and *Datura meteloides* the number of petals and stamens is indefinite, and there is no sharp differentiation between these two floral sets.

The ovary of *Nicandra physalodes* usually consists of five carpels. The abaxial bundle of each carpel is opposite a petal bundle (fig. 4). In the formation of the carpellary vascular system from the stele, there are five outward divergences accompanied by gaps. These divergences are the abaxial bundles. Between each gap the remaining vascular tissue diverges outward and is divided into two strands. Each strand represents an adax-

ial bundle and several small bundles which are anastomosed through the carpellary tissue with the abaxial bundle. The two adaxial bundles which diverge together are for two carpels whose edges represent a septum common to both (figs. 3–5). When these adaxial bundles diverge they are bicollateral, but at more distal levels they are amphicribral.

In *Lycium halimifolium* a complete siphonostele remains after the stamen divergences (fig. 15). Two bundles opposite each other—the two abaxial bundles —diverge outward slightly and are accompanied by gaps. The remaining portion of the cylinder is broken into four equal arcs, each divided periclinally. The bundles of the carpellary walls diverge from the outer portion of each arc, while the inner parts of the four arcs diverge toward the center of the ovary and are the four adaxial bundles (figs. 16, 17). Since these periclinal divisions of the stele are through the xylem, each adaxial bundle has xylem facing the loculus and phloem centrad to the xylem. At more distal levels the bundles become amphicribral.

The carpel bundles of *Atropa belladonna* are derived from a stele in the form of an ellipse (fig. 9). In the two short arcs two bundles diverge outward slightly and are the two abaxial bundles. From the long arcs of the ellipse two large central bundles on each side diverge inward and are the four amphicribral adaxial bundles (figs. 9–11).

The cultivated variety, *Capsicum frutescens* var. *grossum*, has a three-carpellary ovary. The three abaxial bundles diverge from the vascular tissue centrad to three alternate stamen divergences. The inner portions of the other three stamen divergences form three adaxial bundles. At the level at which the three loculi can first be distinguished, the three adax-

ial bundles are in the septa. Then each adaxial bundle is divided into two, one for each adjacent carpel (figs. 33–35).

The carpellary anatomy of *Solanum tuberosum* (figs. 39–41), *S. nigrum* var. *guineense*, *S. pseudocapsicum*, *S. dulcamara*, and *Lycopersicon pimpinellifolium* (figs. 51–53) is similar to that of *Physalis*. In *S. nigrum* and in *L. pimpinellifolium* (figs. 53) the four adaxial bundles form an almost continuous cylinder of vascular tissue. No attempt has been made to interpret the ovary of *S. melongena* var. *esculentum*. Such an explanation is difficult without access to the ancestral type. In the large fleshy gynecia of the cultivated forms the carpellary tissue is very much proliferated and somewhat distorted. The number of carpels is indefinite. Ordinarily there are five to six around a thick central mass of tissue, in which one to two additional carpels may be found. The latter may constitute another set of carpels produced from the axis, which has remained meristematic. Such additional sets of carpels are found in the navel orange, in many "double" flowers, and are not uncommon in the Solanaceae, having been observed in both pepper and tomato. The vascular supply to the carpels of eggplant is derived from the siphonostele remaining after the stamen divergences. At a level just proximal to the formation of each carpel, the two adaxial bundles and the bundles of the carpellary wall diverge inward, so that the position of each carpel becomes first delimited by vascular tissue which is horseshoe-shaped. In the mature carpel there are two semicircles of vascular bundles around each carpel, and the ovules are borne on the placenta and also on what appears to be the carpel wall.

At the level at which the petal and stamen bundles diverge outward in *Datura stramonium*, there are two inward divergences which are directly opposite each other, are accompanied by gaps, and are divided to form four strands of vascular tissue (fig. 57). These inward divergences are the four adaxial bundles, which differentiate as amphicribral bundles in the center of the ovary. After these divergences a continuous cylinder of meristematic tissue is formed (fig. 58). From it are differentiated two abaxial bundles, many small bundles in the carpellary walls, and two somewhat larger and distinct bundles in each of the septa (fig. 59). These last four bundles are probably not different from the bundles in the carpellary walls, but they seem to be much more distinct than the anastomosing bundles in *D. stramonium* and extend up into the basal part of the style. The lower half of the ovary and the fruit appears to have four carpels, resulting from the extension of tissue from the wall of each carpel to the placenta opposite the abaxial bundle. There are no vascular bundles in this tissue, however, and the structure seems to have no morphological significance.

The floral anatomy of the anomalous form, *Solanum rostratum*, resembles most closely that of *Schizanthus pinnatus*. The flowers show a marked tendency toward zygomorphy, with the large lower sepal and stamen differentiating before the other floral parts (fig. 44). This stamen is twice as large as the others (fig. 48). After the divergences to the first three floral sets, the vascular cylinder is divided into two abaxial, two prominent adaxial, and smaller bundles in the carpellary walls (fig. 45). The two adaxial bundles diverge from the periphery of the ovary to its center and fuse to form one adaxial bundle for the two carpels (figs. 46, 47). When the adaxial bundles are diverging, they are bicollateral; after they fuse, the

one bundle is amphicribral. *Solanum* is an extensive genus, and it is possible that evolution has taken place within the genus itself.

While the floral anatomy of the genera studied in Cestreae-Nicotianae and Salpiglossideae is basically like that of *Physalis alkekengi*, individual descriptions are necessary to explain the differences.

After the divergence of the two abaxial bundles in *Nicotiana sanderae*, the remaining carpellary tissue consists of a bundle adjacent to each abaxial bundle and two long segments of vascular tissue opposite each other and on alternate radii from the abaxial bundles. From the middle of each segment an adaxial bundle diverges toward the center of the flower and is divided. There are two amphicribral adaxial bundles for each carpel. In *N. glauca* the two adaxial bundles diverge toward the center of the ovary (figs. 63, 64). For a short distance they are fused to form one double bundle. Distal to this there is one adaxial bundle in the placenta of each carpel (fig. 65).

Each of the five sepal divergences of *Petunia hybrida* trifurcates to form one median and two lateral sepal bundles (fig. 68). These sepal divergences are not accompanied by parenchymatous gaps, but small gaps may be associated with the petal divergences. While all the stamens are fertile, one is smaller than the other four The cylinder remaining after the stamen divergences is divided into four segments—two large ones opposite each other and two small ones on alternate radii (figs. 69, 70). An abaxial bundle and two bundles for the carpellary wall are derived from each small segment. The large one is broken into several bundles for the carpellary wall and an adaxial bundle which diverges toward

the center of the flower. Each adaxial bundle may be double or divided into two bundles (fig. 71). In the mature ovary the adaxial bundles are amphicribral.

In *Nierembergia hippomanica* there are ten sepal divergences, which are not accompanied by gaps. Five of these are the main sepal bundles and five are laterals (fig. 74). When the calyx is separated into lobes, the laterals form a dichotomy with one branch to each adjacent lobe. The petal and stamen bundles are formed in the usual manner (figs. 74, 75). One to three of the five stamens are larger, but all are fertile (fig. 78). The last two stamen bundles to be diverged are opposite the two abaxial bundles. The remaining vascular tissue consists of two segments on opposite radii from the abaxial bundles. From these are diverged the two large adaxial bundles and part of the bundles of the carpellary walls (figs. 76, 77). The remaining bundles of the carpellary walls are lateral divergences from the abaxial bundles. Toward the distal end of the ovary the two adaxial bundles form one bundle, which is then divided into many small ones extending to the base of the style.

Salpiglossis sinuata shows a marked tendency toward zygomorphy and reduction in the number of fertile stamens. There are ten divergences to form five median and five lateral sepal bundles, as in *Nierembergia* (fig. 80). Only three of the ten bundles are accompanied by gaps. One of these is the sepal bundle in the lower lip opposite the staminodium. The other two such bundles are in the upper lip opposite the two large fertile stamens. After the sepal divergences the vascular tissue is usually continuous, although this state is difficult to observe because the lower lip of the flower differentiates at a more proximal level than the upper

part (fig. 81). The petal divergences are not accompanied by gaps. At the level at which the calyx is just diverged from the receptacle, the following bundles can be distinguished in the lower lip: three stamen, two petal, and an abaxial. At the same level the upper lip of the flower consists of one large petal bundle in the uppermost part and a semicircle of vascular tissue toward the center (fig. 81). When the bundles in the lower lip become differentiated, the remaining petal and stamen bundles diverge from the semicircle of vascular tissue in the upper part. There are two large fertile stamens, two small fertile ones, and a staminodium (fig. 84). Two strands of vascular tissue opposite each other remain between the lower and the upper parts of the flower (fig. 82). These diverge to the center and are the two adaxial bundles. At more proximal levels the adaxial bundles appear to be double, but at distal levels and in mature flowers the adaxial bundles are single and amphicribral, with only a slight tendency for the xylem to be separated into two groups by parenchymatous rays. At some levels the two adaxial bundles form an almost complete ring of vascular tissue (fig. 83).

Of all the plants studied, *Schizanthus pinnatus* is the most advanced. It is obviously zygomorphic, and the fertile stamens are reduced to two. In its floral anatomy, however, it still follows the same basic plan of all Solanaceae. The first divergence, the sepal bundle in the lower lip, is not accompanied by a gap. Slightly distal to this first sepal divergence and on either side of it are two petal divergences not accompanied by gaps (fig. 86). These petal divergences trifurcate; the two outer bundles are the lateral ones of the sepals, while the middle one is the petal bundle. Opposite the first sepal bundle there is a divergence to

the most rudimentary of the staminodia. Only the filament of this stamen is formed, and there is rarely any indication of an anther (fig. 88). The divergences to the upper part of the flower are formed in the same manner but depart at more distal levels (fig. 87). The two stamens on either side of the vestigial one are fertile, and their anthers are very large in proportion to the other floral parts (fig. 90). The two uppermost staminodia develop small sterile anthers. After the stamen divergences, the vascular cylinder becomes solid. One to several cells in the center do not stain so dark as the surrounding meristematic vascular tissue. The outer part of this solid vascular cylinder is broken into a circle of small bundles which diverge outward and become the two abaxial bundles and the bundles of the carpellary walls. One abaxial bundle is directly opposite the most rudimentary stamen, while the other is between the two sterile stamens (fig. 88). The inner portion of the vascular cylinder does not change position but becomes the one adaxial bundle common to the two carpels (figs. 88, 89). At the level at which the ovules and placentae appear, one to several vessels become differentiated in the middle of the adaxial bundle. These vessels are the same in number as the large parenchymatous cells in the solid cylinder when it is first formed. The adaxial bundle is single, with no evidence of doubling, but at the top of the ovary this bundle may be divided.

The genera of Solanaceae can be separated into two groups according to the types of fruits produced. *Nicandra, Lycium, Atropa, Physalis, Capsicum, Solanum,* and *Lycopersicon* form berries, while *Hyoscyamus, Datura, Nicotiana, Nierembergia, Petunia, Salpiglossis,* and *Schizanthus* produce capsules. It is inter-

esting to note that, with the exception of *Hyoscyamus*, the more simple flowers develop into berries, while the more complex ones form capsules. Only in the first group is there much anatomical change from ovary to fruit.

In the fruit of *Capsicum frutescens* var. *grossum* the placentae are very much en-

proliferates and almost incloses each seed, but the tissues never actually fuse. The fruit of *S. melongena* var. *esculentum* is produced by enormous growth of the parenchyma of the placentae. The placental tissue of *Lycopersicon pimpinelli-folium* proliferates greatly and almost entirely incloses each of the seeds.
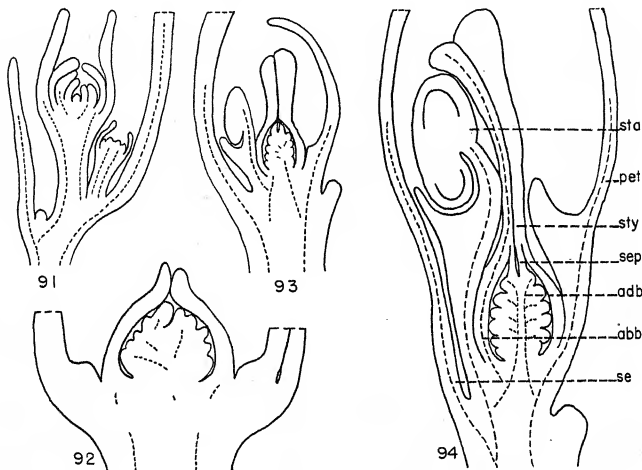
FIGS. 91–94.—Successive stages in floral development. Figs. 91, 93, 94, *Nierembergia hippomanica*. Fig. 92, *Physalis alkekengi*. (*se*, sepal; *sep*, septum; *sty*, style; *pet*, petal.)

larged, but this increase in size does not keep pace with the upward and outward growth of the pericarp and the septa. The placental tissue occupies a relatively small part of the loculi in the lower half of the fruit and is no longer fused with the lower portion of the style. The stylar end of the fruit is unilocular, since the septa are attached only to the pericarp. In *Solanum nigrum* var. *guineense* and in *S. pseudocapsicum* the parenchyma of both the pericarp and the placentae

In floral development the Solanaceous flowers included in this paper follow closely the descriptions of COOPER (4) and WARNER (21) for *Lycopersicon*, URQUHART (17) for *Lycium*, COCHRAN (3) and AUGUSTIN (2) for *Capsicum*, and YOUNG (25) for *Solanum tuberosum*. The sepals, petals, stamens, and carpels are formed in acropetal succession (fig. 91). While in longitudinal section the ovule-bearing tissue of the gynecium appears to be a prolongation of the axis, actually it

is axis plus carpellary tissue. The outer portions of the two carpels arch over the central axile prolongation and finally fuse (fig. 92). Continued elongation of the outer portions of the two carpels forms the style (fig. 93). At the apex of the central prolongation there is a depression, on either side of which the tissue grows upward and fuses with the basal part of the style (figs. 92–94). SATINA and BLAKESLEE (14) describe this occurrence in *Datura*. In the description of *Physalis alkekengi*, at the top of the ovary only the septal tissue remains. Finally the two septa are separated. The tissue which grows upward to fuse with the style is then the tissue of the two septa. The fusion of the septal tissue with the style differs somewhat in the various species studied. In *Hyoscyamus, Physalis, Capsicum, Nierembergia, Salpiglossis,* and *Schizanthus* ovules are formed before this fusion takes place, while in *Datura, Nicotiana,* and *Petunia* this fusion precedes the development of ovules.

Discussion

The syncarpous ovary, according to the Candollean theory, results from the fusion of carpellary leaves. The ovules are borne on the undiverged margins of these leaves, and the axis, if present, serves only as a support for these structures. There are many arguments for and against this theory. Before discussing them, it might be well to define the terminology used in carpel morphology.

The two principal types of placentation are axile and parietal. These terms generally apply only to position and have no morphological significance. Parietal placentation results when the ovules are borne on projections from the carpel wall. When the carpels fuse in the center of the ovary and divide it into as many loculi as there are carpels, the placentae are

axile. PAYER (12), STRASBURGER (16), and GRAY (7) are among those who state that with few exceptions all syncarpous, plurilocular ovaries have axile placentation. "Axial" and "cauline" refer to structures which are morphologically stem, while "foliar" applies to leaflike parts. The confusion in the literature arises from the use of positional terms in a morphological sense.

Among those who disagree with DE CANDOLLE and adhere to the axial theory are PAYER (12), SCHLEIDEN (15), and ST. HILAIRE (WORSDELL, 24). PAYER states that the "axile part of a syncarpous ovary constitutes the placentae and bears the ovules, while the carpellary leaves constitute the walls of the ovary." SCHLEIDEN states: "In all families with plurilocular ovaries one can easily follow the origin of the placentae which come from the axis." ST. HILAIRE also thinks that "the placenta is a continuation of and represents the axis." Between the axial and the appendicular theories is HAYWARD (9), who explains: "In the axile type of placentation, the placental tissue lies at the center of the ovary and may be foliar, or in part foliar and in part cauline."

Outstanding followers of the Candollean theory are VAN TIEGHEM (18, 19), HENSLOW (10, 11), EAMES (5), and ARBER (1). VAN TIEGHEM and HENSLOW are particularly helpful because they give definite criteria for interpreting the nature of the placentae. The following quotations are taken from HENSLOW. The first one (10) explains the views of VAN TIEGHEM, while the second one (11) gives the ideas of HENSLOW on the subject.

Van Tieghem in his investigations upon the anatomy of the pistil regarded the circle of vascular cords which have their trachae on the inner side, i.e. facing the medulla, and the

phloem on the outside of it, i.e. facing the cortex, as indicating the axis; but when the circle is broken up and these two elements of the cords are altered or reversed in position as they often are in the placentas of a carpel, he pronounced them to be "foliar."

The point, then, at which carpellary cords branch off from the common stem in the first case may be regarded as marking the termination of their axial character; and in the latter case, at the separation of the parts of the "horse-shoes" to form groups of threes. With regard to those cords which become marginal and placentary, it is important to notice the position of their spiral vessels. If they are situated on the side of the cord nearest the medulla, the cord may generally be regarded as axial; if they are on the other side, i.e. nearest the ovary-cell, and if in transverse sections they exhibit intermediate positions, in which they are central or scattered irregularly within the phloem, they are then marginal and placentary.

When floral parts are undiverged, the vascular anatomy seems to be the most valuable criteria for determining the origin of the parts involved. In this paper, therefore, the interpretation of VAN TIEGHEM and HENSLOW is used. If the adaxial bundles retain their stem-like character, with the xylem facing the center of the flower, the structures will be considered as cauline; if the xylem is facing the loculus, or if the bundles are amphicribral, the structures will be considered as foliar.

As for the literature on the Solanaceae, PAYER (12), VIDAL (20), and RENDLE (13) describe the placentae as axile. In *Lycopersicon esculentum* var. *cerasiforme*, WARNER (21) explains the placentae as follows: "Apparently it is from the central more or less columnar axis that ovules arise. Thus they may be thought of as cauline in origin, or perhaps, the surface of this axis may be regarded as an undiverged portion of the sporophyll, in which case the ovules would be regarded

as foliar." URQUHART (17), working with *Lycium halimifolium*, states: "Although the placental structure is at this stage continuous with the carpellary tissue, it is largely cauline in origin. The ovules may be regarded as arising from cauline, axile placentae rather than foliar ones."

VIDAL (20) studied several species of the Solanaceae and concluded that in all of them the axis forms a thick septum raised to the summit of the ovary. In summarizing his work, VIDAL states: "The Solanaceae are characterized by a very well developed, parenchymatous axis raised to the summit of the ovary."

The most comprehensive paper on floral anatomy in the Solanaceae is by GRELOT (8). His emphasis is somewhat different from the present work, but on the whole the results agree. Concerning the adaxial bundles, he says: "If a certain number of bundles converge toward the center of the placentae and unite side by side to form a cylinder, the latter delimits a true pith whose role is different from that of the exterior parenchyma of the cylinder. One perceives then that the bundles take the same orientation as that in the stem." SATINA and BLAKESLEE (14), who worked with periclinal chimeras obtained in *Datura*, state: "The initiation and development of the carpel wall, septa, and placentae suggest that they are axial and not foliar in origin."

Of a rather different opinion is GOEBEL (6). He describes the development of the carpels as follows:

The process is exactly the same where we have in each loculus a many-ovuled placenta developed as in Solanaceae and Scrophulariaceae. The ovary in its upper part is unilocular with two parietal placentas. . . . . The carpels use up entirely the torus, and form to a certain extent a double sole, the septal wall. The margin of the cup of the ovary shows an increased growth at the points corresponding to

the apices of the carpels and the lateral parts raise themselves somewhat at the position of concrescence and there form the parietal part of the placenta. Beyond this the question of how far the flower-axis is drawn into the formation of the ovary is of quite subordinate importance.

The floral anatomy of the Solanaceae demonstrates that the placentae are usually be distinguished. GRELOT's contention that such a cylinder is proof of the cauline nature of the placental bundles seems unfounded. The carpellary walls, the septa, and the ovule-bearing portions of the placentae are foliar in origin. The central fleshy part of the ovary is parenchymatous stelar tissue.

TABLE 1

Species	Type of flower	Stamens	Adaxial bundles	Fruit
Nicandra physalodes Atropa belladonna Lycium halimifolium	Actinomorphic	All fertile	Two per carpel	Berry
Hyoscyamus niger	Slightly zygomorphic	All fertile	Two per carpel	Capsule
Capsicum frutescens Solanum nigrum S. melongena S. dulcamara S. pseudocapsicum S. tuberosum	Actinomorphic	All fertile	Two per carpel	Berry
Solanum rostratum	Zygomorphic	1 large 4 small}fertile	One for 2 carpels	Berry
Lycopersicon pimpinellifolium	Actinomorphic	All fertile	Two per carpel	Berry
Datura stramonium D. meteloides	Actinomorphic	All fertile	Two per carpel	Capsule
Nicotiana sanderae	Slightly zygomorphic	All fertile	Two per carpel	Capsule
N. glauca	Slightly zygomorphic	All fertile	Two for 2 carpels	Capsule
Petunia hybrida	Slightly zygomorphic	4 large}fertile 1 small	Two double bundles for 2 carpels	Capsule
Nierembergia hippomanica	Slightly zygomorphic	1-3 large}fertile 4-2 small	Two for 2 carpels	Capsule
Salpiglossis sinuata	Zygomorphic	2 large}fertile 2 small 1 sterile	Two for 2 carpels	Capsule
Schizanthus pinnatus	Zygomorphic	2 large fertile 2 small sterile 1 vestigial	One for 2 carpels	Capsule

foliar in origin. In all the species studied the adaxial bundles in the older flowers and fruits are amphicribral and accompanied by gaps in the vascular cylinder. This applies even to *Schizanthus pinnatus* and *Solanum rostratum*, which have only one adaxial bundle. The occurrence of the adaxial bundles as a cylinder in the center of the ovary was noted in *Solanum nigrum, Lycopersicon pimpinellifolium,* and *Salpiglossis sinuata,* but the vascular tissue does not resemble an amphiphloic siphonostele and the individual amphicribral bundles can

In the Solanaceous flower the margins of the carpels are undiverged from the stem. At first there is conjoint growth of stem and carpels, but in the more mature flower the apical region of the stem ceases its meristematic activity. The exception is found in such plants as tomato, eggplant, and pepper, where growth continues to form a second fruit. Just how much of the ovule-bearing prolongation of the ovary is cauline and how much foliar would be difficult to prove. The adaxial bundles are foliar, and in longitudinal section they probably limit the

inner bounds of the carpels. The placentae are axile in position and foliar in origin. The Solanaceous flowers described in this paper can best be interpreted by the appendicular theory of DE CANDOLLE.

In general, the floral anatomy furnishes additional evidence of the validity of WETTSTEIN's arrangement of the genera. It would seem better to place the Hyoscyaminae at the end of the Solanaceae because the flowers of *Hyoscyamus* are slightly irregular and the fruit is a capsule. Among the Cestreae-Nicotianae and the Salpiglossideae there is a gradual reduction in the number of adaxial bundles per carpel. The phylogenetic tendencies of the Solanaceae described in this paper are summarized in table 1.

Summary

The floral anatomy of fourteen genera and twenty-one species of the Solanaceae was examined. The arrangement of the genera is according to WETTSTEIN in ENGLER and PRANTL's *Die Natürlichen Pflanzenfamilien*. In the order of their complexity, these genera include *Nicandra, Lycium, Atropa, Hyoscyamus, Physalis, Capsicum, Solanum, Lycopersicon, Datura, Nicotiana, Petunia, Nierembergia, Salpiglossis,* and *Schizanthus*. The following evolutionary tendencies were noted: actinomorphy to zygomorphy; reduction of fertile stamens from five to two; and reduction in the number of adaxial bundles from two per carpel to one for two carpels. Despite these variations, the floral anatomy follows the same basic plan in all the species studied. The siphonostele generally becomes continuous after the divergences to each of the first three floral sets, and the carpellary bundles are formed from the remaining vascular tissue. Because the adaxial bundles are amphicribral and accompanied by gaps in the vascular cylinder, they are foliar rather than cauline. The placentae are axile in position, while the carpel walls, the septa, and the ovule-bearing portions of the placentae are foliar in origin. The central parenchymatous part of the ovary is stelar, and the carpels are undiverged leaves.

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INVESTIGATIONS ON RUBBER-BEARING PLANTS: I. PROP-
AGATION OF TARAXACUM KOK-SAGHYZ
BY MEANS OF LEAF CUTTINGS[1]

PAUL R. GORHAM AND MARGARET L. LANDES[2]

Introduction

Interest in obtaining polyploid deriva-
tives from diploid clones of *Taraxacum
kok-saghyz* Rod. prompted an experiment
to propagate leaves from colchicine-
treated root cuttings which gave indica-
tions of possessing an increased number
of chromosomes. Since material from this
source was limited, it was decided to
carry out a series of preliminary experi-
ments using diploid leaves. In a compari-
son of propagation by means of root,
crown, and leaf cuttings, MYNBAYEV (3)
found the last method too unsatisfactory

to warrant further consideration. How-
ever, BOWDEN (1) succeeded in obtaining
a few plants from leaf cuttings of the
Russian dandelion. NAVASHIN and GER-
ASSIMOVA (5) reported that diploid and
tetraploid leaves from this plant can be
propagated, but in the absence of sub-
stantiating data it was not apparent
what degree of success might be ex-
pected.

HAGEMANN (2) has reviewed, up to
1932, most of the extensive literature on
propagation by means of leaf cuttings,
while SWINGLE (7) has summarized pub-
lications up to 1940. Many of the original
papers report results that are frequently
inconclusive because of the small num-

bers of leaves used. As HAGEMANN emphasizes, negative results do not necessarily mean that propagation by this method is impossible. A few of these papers contain references to other *Taraxacum* species. HAGEMANN planted eighteen leaves of *T. officinale*, all of which died within 27 days without regenerating roots or shoots. STINGL (6) used forty leaves of *T. officinale* Weber, but these died within 3 days. NAYLOR (4) reported negative results with *T. laevigatum* (Willd.) DC.

Material and methods

For the experiments relating to the temperature of the cutting-frame and treatment with KNO₃, leaves were taken in January from clones of vigorously growing young root cuttings started in flats 2 months before. The material used in the comparison of regeneration of leaves from young and mature plants is described with the experiment. Plants used in the tests with indole-3-butyric acid, naphthaleneacetic acid, asparagine, glycine, *p*-aminobenzoic acid, and glucose were chosen early in March from several clones which had been propagated by means of root cuttings 6–8 months before. These plants had reached 5-inch pot size and had one or two rosettes with twenty to thirty leaves each. The leaves of *Taraxacum latilobum* DC. were taken in April from a plant brought into the greenhouse 8 months before from the collection of Arctic dandelions at the Dominion Arboretum and Botanic Garden, Ottawa.

Young tender leaves in the center of the crown and old yellowed leaves at the periphery were found to root with difficulty and therefore were not tested. With the exception of the treatments employing talc dust, leaves were removed from the parent plant by pulling them away from the crown under water. After rubbing the base between the fingers to remove, as far as possible, any adhering crown tissue, the leaves were stood in the aqueous test solutions. After a stated period they were set in sand in a greenhouse cutting-frame and clipped to a height of 3 inches, in order to reduce transpiration. The temperature of the cutting-frame was maintained at approximately 68°F. The glass top of the frame was covered with paper to screen out direct sunlight. Relative humidity was maintained at a moderate level by frequent watering. The top was slightly raised at night, fully raised during the day. Survival after 10 days was noted and dead leaves were discarded. After 20 days, and again after 30 days, rooted leaves were potted in equal parts sand and manurial compost in 2½-inch pots and placed on a bench in the same house. All unrooted leaves were then discarded. The base of the rooted leaf was planted ½ inch or less in depth, since it was found that shoots form readily only when the leaf bases are just below the soil line. This confirms a similar observation made by NAVASHIN and GERASSIMOVA (5).

Experimentation

The advantages of removal of leaves under water over removal in air is demonstrated by table 1. Excessive wilting is obviated, and improved survival during the first 10 days as well as improved rooting and regeneration result.

Four hundred and fifty leaves from ten clones were divided equally among the shaded cutting-frame at 68°F. as described and two electrically heated frames regulated at 72° ± 2°F., one covered and the other uncovered. At the higher temperature all the leaves died in the covered frame within 10 days and in the uncovered frame within 15 days. At

the lower temperature fifteen leaves in two clones rooted.

Roots of two clones grown in the field for one season from root cuttings were potted in manurial compost December 1. Simultaneously, root cuttings from the same material were started in flats in equal parts of sand, compost, and peat.

TABLE 1

EFFECT OF REMOVAL OF LEAVES IN AIR COMPARED WITH REMOVAL UNDER WATER. TOTALS ARE FROM CONTROL LEAVES OF TWO CLONES COMMON TO TWO DIFFERENT EXPERIMENTS

TREATMENT	No. LEAVES	PERCENTAGE			
		Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Air.........	32	53	9	13	0
Water......	64	67	25	38	13

After 53 days the potted plants had abundant leaves and were in full bloom, without fruit. The root cuttings had numerous smaller leaves, with flower buds appearing. The regenerative capacities of leaves from young and from mature plants of these two clones were compared. Root cuttings of clone 93.21 had a higher percentage regeneration than root cuttings of clone 93.38, but this difference was not evident in the regeneration of the leaf cuttings (table 2). Initial survival of leaves from young plants is somewhat better than that of leaves from mature plants, but the production of roots and shoots does not differ appreciably.

Sixty leaves from three clones had $\frac{1}{8}$ inch removed from the base by cutting under water with a razor. These basally clipped leaves regenerated to the same extent as the controls. The leaf tips of 170 leaves from three clones were tested also. The initial survival of these tips was low and all were dead within 20 days.

THIMANN and POUTASSE (8) found that treatment with KNO_3 improved rooting of *Phaseolus* leaves by supplying nutrient to the severed blade. Leaves of *T. kok-saghyz* from eight clones, treated with KNO_3 at a concentration of 500 mg. per liter, had a slightly lower percentage regeneration after 70 days than had the controls (table 3). Roots produced by the treated leaves were only two-thirds as long as those produced by the untreated leaves.

Indole-3-butyric acid in talc, at concentrations of 1000, 2000, and 5000 p.p.m., was tested for its effects on regeneration by dipping the bases of the leaves from each of five clones in the

TABLE 2

COMPARATIVE REGENERATIVE ABILITY OF LEAVES FROM ACTIVELY GROWING YOUNG AND MATURE PLANTS

CLONE	REGENERATION OF ROOT CUTTINGS (%)	TYPE	No. LEAVES	PERCENTAGE			
				Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
93.21.........	89	Mature	258	83	19	19	5
		Young	206	88	19	23	1
93.38.........	35	Mature	82	50	11	11	4
		Young	48	71	21	21	4

dusts just prior to planting. There were slight indications of toxicity at 5000 p.p.m. and of improved regeneration at 1000 and 2000 p.p.m. (table 4).

Naphthaleneacetic acid at a concentration of 50 mg./l., and a solution containing 250, 3, and 1 mg./l. of asparagine, glycine, and *p*-aminobenzoic acid, respectively, were both tested by standing leaves from each of four clones in the solutions for 3 hours prior to planting. Although there was lowered regeneration following the first treatment and improved initial survival and rooting following the second (table 5), natural variability might well account for differences of this magnitude.

Approximately equal numbers of leaves from each of five clones were treated 3 hours in seven test solutions. These solutions comprised various concentrations and combinations of *p*-aminobenzoic acid, indole-3-butyric acid, asparagine, and glucose (table 6). Initial survival was surprisingly uniform for all treatments. Neither rooting nor regeneration was improved by treatment with indole-3-butyric acid, asparagine, or glucose. Response to *p*-aminobenzoic acid concentrations was inconsistent, but in general it did not seem to improve regeneration.

Complete records were kept of the re-

sponses of leaves according to their clonal origin, since root cuttings from different clones have different regenerative capacities (unpublished data, and table 2). That such is the case for leaves is apparent from table 7, where the regenerative ability of leaves from six different clones is compared when untreated and

TABLE 3

EFFECT OF TREATING LEAVES FOR 20 HOURS IN 500 MG./L. KNO₃. TOTALS ARE FROM EIGHT CLONES

TREATMENT	No. LEAVES	PERCENTAGE		
		Survival 10 days	Rooted 20 days	Regenerated 70 days
KNO₃..........	166	51	24	6
Water (control)..	176	64	17	8

when all treatments are combined. These data serve to illustrate the numbers of leaves needed in order to establish with reasonable accuracy the regenerative ability of particular clones. Within clone 93.27, regeneration from actively growing parent plants was particularly noteworthy but was lower when leaves were subsequently tested from inactively growing plants. However, differences in environment between tests may partially account for the observed decrease.

TABLE 4

EFFECT OF DIPPING LEAF BASES IN INDOLE-3-BUTYRIC ACID-TALC MIXTURES. APPROXIMATELY EQUAL NUMBERS OF LEAVES FROM EACH OF FIVE CLONES WERE TREATED AND RESULTS TOTALED

TREATMENT	CONCENTRATION (P.P.M.)	No. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Indole-3-butyric..........	5000	134	37	5	15	2
Indole-3-butyric..........	2000	92	47	14	17	3
Indole-3-butyric..........	1000	113	45	12	16	4
Talc (control)............	....	121	55	7	12	1

Forty leaves of *T. latilobum* were tested in the same manner as those of *T. kok-saghyz*. At the end of 10 days, twenty-two leaves survived. Within 30 days, one leaf had a shoot but no roots. Within 60 days, two of the leaves had short roots and were potted in manurial compost. Each of the six unrooted leaves which still survived had several calluses, some as large as peas. A week after potting, one of the rooted leaves produced a shoot which later developed into a vigorous plant.

Discussion

In isolated leaves of *Taraxacum kok-saghyz*, shoots arise from leaf-borne roots (type III, HAGEMANN's classification). The base of the leaf cutting first forms an inconspicuous callus which proceeds to

TABLE 5

EFFECT OF 3-HOUR TREATMENT WITH NAPHTHALENEACETIC ACID, AND WITH A
COMBINATION OF ASPARAGINE, GLYCINE, AND *p*-AMINOBENZOIC
ACID. TOTALS ARE FROM FOUR CLONES

TREATMENT	CONCEN-TRATION (MG./L.)	No. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Naphthaleneacetic........ Asparagine.............. Glycine................ *p*-Aminobenzoic........	50 250 3 1	116 128	59 77	27 33	33 39	3 7
Water (control)...........	....	121	62	22	30	8

TABLE 6

EFFECT OF TREATING APPROXIMATELY EQUAL NUMBERS OF LEAVES FROM EACH OF FIVE
CLONES FOR 3 HOURS IN SOLUTIONS OF *p*-AMINOBENZOIC ACID, INDOLE-3-BUTYRIC
ACID, AND COMBINATIONS OF THESE WITH ASPARAGINE AND GLUCOSE

TREATMENT	CONCEN-TRATION (MG./L.)	No. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
p-Aminobenzoic..........	0.1	87	55	15	15	4
p-Aminobenzoic..........	1	86	57	14	14	1
p-Aminobenzoic..........	10	86	57	24	24	11
Indole-3-butyric..........	50	85	60	18	21	8
p-Aminobenzoic.......... Asparagine.............. Indole-3-butyric..........	1 75 50	87	62	21	23	4
p-Aminobenzoic.......... Asparagine.............. Indole-3-butyric.......... Glucose.................	1 75 50 25,000	85	57	26	27	8
p-Aminobenzoic.......... Asparagine.............. Indole-3-butyric..........	0.1 75 50	87	67	29	23	5
Water (control)..........	....	85	58	28	29	6

TABLE 7

COMPARISON OF REGENERATIVE ABILITY OF LEAVES FROM SIX CLONES
WHEN UNTREATED AND WHEN ALL TREATMENTS ARE COMBINED

CLONE		No. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
93.27*	(Un.)†	44	61	32	34	27
	(All)	322	63	26	30	21
93.42	(Un.)	21	57	33	33	0
	(All)	334	52	11	18	2
93.24	(Un.)	63	65	19	32	2
	(All)	535	61	23	29	2
93.31	(Un.)	43	58	23	26	7
	(All)	178	66	39	40	7
253.3	(Un.)	9	67	11	11	0
	(All)	120	40	3	4	0
93.22	(Un.)	44	48	27	27	5
	(All)	226	54	25	25	1
93.27‡		70	41	7	11	6

* Leaves from actively growing plants.
† Un., untreated; All, all treatments combined.
‡ Leaves from plants in inactive state of growth.

FIGS. 1–4.—Fig. 1, leaf cutting showing calluses at ends of vascular strands. Figs. 2, 3, roots arising from calluses. Fig. 4, developing shoot (parent leaf thickened). Magnification of fig. 1 twice that of figs. 2–4.

develop most noticeably about the severed ends of the vascular strands (fig. 1). From these callus swellings one to several roots arise (fig. 2) which elongate (fig. 3) and frequently become much-branched. Shoots arise from the callus swellings at the apex of the root (fig. 4); exceptionally shoots arise on the root below this point.

Those leaves which survive the first 10 days have callused successfully. With favorable environmental conditions, mortality during the next 10 days is low. During this time the greater part of the root initiation occurs. Thereafter, the amount of root initiation taking place is so limited (table 7) as to make it impractical to retain callused, unrooted leaves any longer. Bits of crown tissue which may remain attached to the leaf base are not essential for regeneration, since leaves with the basal portion removed also regenerate. This ability evidently decreases toward the leaf tip, since the distal portions of leaves were incapable of regeneration.

In a very few instances shoots may arise from unrooted callus swellings, but these have low viability. Concurrent development of root and shoot may take place before the leaves are potted on the twentieth day, but most shoots appear later. Parent leaves which persist become thickened and curled and can still be distinguished when the rosette of the new plant has attained considerable size. Death of the parent leaf is not prejudicial to shoot production if the root system remaining is well established. In fact, the upper portion of the roots may become considerably thickened before shoots appear.

More than one shoot may arise from a rooted leaf cutting. This becomes most evident upon death of a parent leaf, when the separated callus swellings continue to regenerate independently. As many as four regenerated plants have been observed to originate from a single leaf cutting.

Leaves from actively growing plants vary in their capacity to regenerate, depending on their clonal origin. Within a particular clone the state of maturity of the parent plant has little effect on the regenerative capacity of the leaves, provided it is in an active state of growth. Leaves from mature plants of the same clone have a higher or lower regenerative capacity, depending on whether the plants are growing vigorously or not. Moreover, the outer senescent and inner juvenile leaves of a whorl do not regenerate so well as do those in between. Actively growing, physiologically mature leaves seemingly possess factors facilitating regeneration to a greater degree than do leaves which are inactively growing, juvenile, or senescent; and clones therefore differ from one another in the amounts of such factors each is potentially able to produce. None of the substances tested stimulated regeneration or helped to correct deficiencies of these factors.

The foregoing experiments demonstrate the rather limited success to be expected in the propagation of *T. kok-saghyz* by means of leaf cuttings. Such practice is warranted only under special circumstances, such as the establishment of polyploid clones that might be compared with the diploid clones from which they were derived. This would avoid the difficulties inherent in diploid-polyploid comparisons between heterogeneous seedling populations. Two tetraploid plants have been obtained by the successful regeneration of leaves from colchicine-treated root cuttings of one clone, providing evidence that such leaves are capable of being propagated.

Summary

1. Factors considered essential in the propagation of leaves of *Taraxacum kok-saghyz* Rod. are: (*a*) removal under water, (*b*) moderate humidity and a temperature of approximately 68°F. in the cutting-frame, and (*c*) potting with the rooted leaf base just below the soil line.

2. Under the conditions of the experiments, treatments with indole-3-butyric acid, naphthaleneacetic acid, glucose, asparagine, glycine, *p*-aminobenzoic acid, and potassium nitrate, in various concentrations and combinations, failed to improve regeneration.

3. Leaf regeneration of *T. kok-saghyz* was by means of shoots arising from leafborne roots.

4. Clones differed in their ability to regenerate. The observed variation ranged from 0% to 27%. The condition of growth at the time of testing, rather than the age of the parent plant, seemed to determine variation within a clone.

5. One out of forty leaves of *T. latilobum* DC. regenerated.

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EFFECTS OF SOAKING WITH INDOLEBUTYRIC ACID ON ROOT DEVELOPMENT AND SURVIVAL OF TREE SEEDLINGS

T. E. MAKI AND HUBERT MARSHALL[1]

Introduction

The last decade has witnessed a phenomenal increase in our knowledge of growth-regulating substances and in our ability to use them to regulate plant behavior. These substances are used today for several commercial purposes, including the rooting of cuttings of woody species heretofore difficult or impossible to root by conventional methods. The widespread use of growth regulators on cuttings naturally has awakened interest in stimulating the production of new roots on transplant stock. Such a need is felt acutely in forest planting, since the nature of the undertaking makes it uneconomical to transplant seedlings to the field with balled and relatively intact root systems. In handling bare-rooted stock, a large portion of the root system is lost in lifting from the nursery bed, or is pruned off to facilitate rapid planting in the field; in consequence, death of seedlings may result from critically reduced water absorption. If growth-regulator treatments could be perfected which would stimulate rapid production of new roots immediately after planting, considerable loss in forest plantations might be avoided.

Studies of root formation on cuttings have shown that growth regulators stimulate the initiation of root primordia, which subsequently elongate under the influence of other factors. Other studies have shown that growth regulators, in concentrations which initiate root pri-

mordia, are likely to inhibit the growth of both shoots and roots. The problem, then, becomes one of devising a schedule of treatments which will bring about the initiation of primordia on the old roots but which will not inhibit their subsequent elongation. Several investigators have reported stimulated root development on transplants, but, for the most part, they have given few or no data on subsequent survival. The study here reported was designed to clarify conflicting reports in the literature and to determine whether the root stimulation frequently reported actually can be translated into increased survival.

Review of literature

Several investigators have studied the effects of vitamins on woody plants. BENSEND (2) grew jack-pine seedlings in sand irrigated with a balanced nutrient solution to which thiamin or niacin was added at periodic intervals. After 80 days no differences in size or weight were found, and analysis of the tissue showed that controls contained as much thiamin as treated seedlings. Similarly, PARKER et al. (7) found that young Valencia-orange trees planted in soil with bare or balled roots and with adequate water and mineral elements failed to respond to either thiamin or niacin added to the irrigation water.

On the other hand, SWARTLEY and CHADWICK (12) dusted groups of ten rooted cuttings of *Lonicera fragrantissima* with Transplantone no. 1 (a commercial talc preparation of growth regulators and vitamins in undisclosed proportions), and with talc dusts of nico-

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tinic acid 1–1000 and thiourea 1–20,000, before planting in beds in a slat-house or in pots in a coldframe. Two months later all treatments (especially nicotinic acid) in the slat-house had improved top growth, but no response was noted in the coldframe. In a second experiment, lots of ten seedlings of *Malus sieboldi arborescens* were root-dusted with talc mixtures of nicotinic acid 1–500 and 1–1000, indolebutyric acid 1–5000, and Transplantone no. 1. Indolebutyric acid and Transplantone produced clusters of new roots but nicotinic acid was ineffective. Lastly, lots of twenty-five to seventy-five seedlings of *Juniperus virginiana* and *Picea glauca* were watered with Transplantone, either once or three times, the latter treatment being superior. On *Juniperus*, both number of new roots and survival were increased, but *Picea* failed to respond.

Several reports have appeared on the use of growth regulators on coniferous stock. PLANK (8) soaked groups of ten slash-pine seedlings for 24 hours in aqueous solutions of indolebutyric acid ranging between 10 and 80 mg. per liter. One year after planting, root development showed stimulation from the lower concentrations. In a more elaborate experiment, FOWELLS (4) grew groups of twenty ponderosa-pine seedlings in nutrient tanks or pots of soil after soaking the roots for 24 hours in 5, 25, or 50 mg. of indoleacetic acid or thiamin per liter of water. After 3 months in the tanks, seedlings soaked in 5 mg./l. indoleacetic acid produced significantly longer roots than did controls. Seedlings planted in soil and treated with 50 mg./l. indoleacetic acid produced significantly more secondary roots than did seedlings treated in any other manner. No other differences were found, and FOWELLS concludes that the results were not encour-

aging. A similar conclusion was reached by ZACH *et al.* (14) after treating a number of coniferous species with indolebutyric acid and planting them in a nursery and on an adverse site in the field.

Somewhat more work has been reported on hardwoods. LEFEVRE (6) applied talc dusts of indolebutyric and indoleacetic acids in concentrations of 1-10,000 and 1-100,000 to the roots and grafts of grapevines, with subsequent improvements in root development and quality of callus. CHADWICK (3) soaked the roots of 3-year-old plants of *Viburnum dilatatum* and *Cotoneaster divaricata* for 18 hours in aqueous solutions containing 20, 50, and 100 mg./l. of indoleacetic, indolebutyric, or phenylacetic acid. After 69 days, *Viburnum* treated with 20 mg. of indolebutyric acid had developed seven times more new roots than controls. *Cotoneaster*, after the same length of time, had responded most favorably to the same substance at 50 mg./l. A similar response was obtained by adding 10 or 20 mg. of the acids to the soil in 5-inch pots. Applying the growth regulators by dipping the roots into emulsified paraffin containing the acids was not successful.

TILFORD (13) lifted seedlings of American elm, hard maple, black oak, pin oak, black walnut, white pine, Norway spruce, and American arborvitae in January and soaked the roots for 48 hours in growth regulators at 25 mg./l. The seedlings were potted and remained in the greenhouse until April, when all except white pine and Norway spruce showed definite root stimulation. Indolebutyric acid produced the greatest response. In a second experiment, American-elm and red-oak saplings were root-pruned, treated, and planted in the field. Dipping in, or watering with, growth

regulators, or smearing the cut ends of the roots with lanolin mixtures, failed to induce response; but soaking the roots for 48 hours in 40 mg./l. indolebutyric acid (the better substance) or indoleacetic acid more than doubled the number of new roots by the end of the first growing season.

At the Rubber Research Institute of Malaya (1), budded stumps of rubber trees were lifted from the nursery, root-pruned about 18 inches below the collar, and soaked for 16 or 24 hours in a dilute solution of a commercial growth regulator covering only the lower $1\frac{1}{2}$ inches of the taproot. Substantial increases were obtained in the number of stumps taking root and in the total root production.

SMITH and ROMBERG (10) devised a method of treating pecan transplants with growth regulators which they regard as superior to either soaking or localized lanolin treatment. The method consists of soaking toothpicks in 95% alcohol in which indolebutyric acid has been dissolved in such concentration as to allow the picks to absorb 4 mg. of the acid. From one to several picks are then inserted into holes bored transversely through the larger roots. The acid diffuses into the surrounding tissues, where it locally stimulates root formation. Using this method, treated 5- and 7-year-old pecan trees developed, by the end of the first growing season, about three times as many new roots and four times as much new root length as untreated controls (9). In later experiments (11), the dry weight of new root growth on 5-year-old pecan trees was increased in one test from 8.2 gm. on controls to 15.6 gm. on treated plants, and in another test from 4.8 gm. to 10.0 gm. Equally striking results were obtained with large trees having trunk circumferences of approximately 14 inches. Al-though none of the tests was carried out with the primary object of obtaining survival data, wherever mortality occurred controls suffered more than treated trees. GOSSARD (5) made an additional test of the technique of SMITH and ROMBERG. He found that treated pecan trees produced significantly more root growth than controls but significant differences in survival and shoot growth were not obtained.

Investigation

PRELIMINARY EXPERIMENTS, 1942

Although the toothpick technique of SMITH and ROMBERG may be of value in treating orchard or horticultural stock, it obviously would be impracticable with the small-sized stock commonly used in forest planting. Of the other treatments, dusting and soaking the roots seemed to offer promise, particularly in view of TILFORD's work (13). Accordingly, an exploratory study[2] was carried out in 1942 to test the effects of growth regulators on a wide variety of plant materials. During late spring and early summer, seedlings or small plants of twenty-six species of evergreen and deciduous trees and shrubs were treated by soaking the roots for 24 hours in aqueous solutions of indolebutyric acid in concentrations ranging from 0 to 1000 mg./l. Twenty plants per treatment were used. Immediately after treatment, the stock was planted in a plowed field according to a statistically acceptable design. At the end of the summer the plants were dug and determinations made of total weights, root quality and volume, and amount of new top growth. In general,

concentrations of 250 mg./l. and higher resulted in poorer root and shoot growth and considerable increase in mortality. Concentrations below 250 mg./l. produced variable results. With nursery-grown stock with adequate survival, for example, significant increases in root inhibited shoot growth in proportion to concentration, and the higher levels unquestionably were harmful, in some instances causing leaves to fall prematurely, even though stem and roots remained alive (fig. 1). Among species suffering heavy mortality in the con-
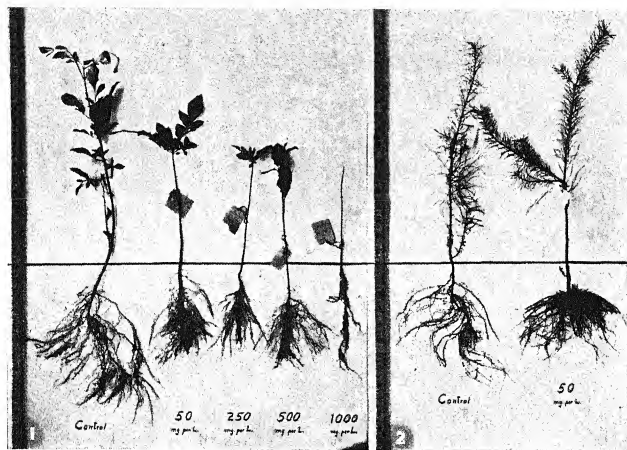
FIGS. 1, 2.—Fig. 1, white-ash seedlings, 1 year old when treated, showing effect of indolebutyric acid gradient on shoot and root development 2 months after treatment. Soaking roots in the acid caused inhibition of shoots, even in lower concentrations. On an absolute basis, treated seedlings had smaller root volumes; on a basis relative to shoot growth, the reverse was frequently true. Fig. 2, jack-pine seedling on right treated by soaking roots for 24 hours in indolebutyric acid at 50 mg./l. This treatment was generally not beneficial, but occasional seedlings responded with greatly stimulated root development.

volumes were found on loblolly (*Pinus taeda* L.) and shortleaf (*Pinus echinata* Mill.) pines, but no beneficial responses occurred with red (*Pinus resinosa* Ait.), white (*Pinus strobus* L.), and pitch (*Pinus rigida* Mill.) pines, nor with white ash (*Fraxinus americana* L.), tuliptree (*Liriodendron tulipifera* L.), and paper birch (*Betula papyrifera* Marsh.). In many species, root soaking trols, only a few treatments brought about increases in survival. Altogether, these results were not particularly encouraging. However, many examples of root stimulation on individual seedlings were observed (fig. 2). While the marked responses of these seedlings were not great enough or frequent enough to result in significant increases for their treatment groups as a whole, they did

provide hope that root growth (and probably survival) could be increased if the factors causing the individual responses could be determined.

PRELIMINARY EXPERIMENTS, 1943

The following spring an effort was made to determine with greater precision the effects of growth regulators on tuliptree and loblolly pine. It was felt that if definite prescriptions could be worked out for a few species, the knowledge gained would greatly facilitate adaptation of such treatments to other species. Accordingly, groups of sixty loblolly pine and tuliptree seedlings were root-soaked for either 4 or 22 hours in aqueous solutions of naphthaleneacetic acid, naphthalene acetamide, indolebutyric acid, or all four combinations of these three substances at 30, 90, or 270 mg./l. This design departed from the earlier study primarily in the reduced number of species, the lower concentrations, the addition of a short soaking period, and the comparison of both single growth regulators and mixtures in factorial combinations. Detailed studies of root development were not made, but survival tallies showed consistent increases in mortality resulting from almost all treatments. Indolebutyric acid was less toxic than the other substances and at the lowest concentration was not harmful.

Tuliptree was used both years and failed to benefit in either root growth or survival. Loblolly pine responded in 1942 with stimulated root development but showed no benefits in 1943. These tests gave little promise that soaking the roots in solutions of the compounds would substantially reduce mortality of forest planting stock. However, other tests in 1943 with growth regulators applied to hardwood stock in common storage showed again that under the proper circumstances root growth can be greatly stimulated. Accordingly, a small experiment was begun in May, 1944, to study in greater detail the effects of indolebutyric acid on the root development of one pine and one hardwood species.

MAIN EXPERIMENTS, 1944

MATERIAL AND METHODS.—One-year-old loblolly pine was selected as a test species because it had been used in both preliminary studies and had shown favorable root response in 1942. One-year-old eastern red oak (*Quercus borealis maxima*) was chosen as the other species because closely related species of the black-oak group had responded well in the 1942 study and in TILFORD's (13) earlier experiments.

The stock was carefully graded to a uniform size, root-pruned, and tied into groups of ten seedlings for determination of root volumes by water displacement in a graduated cylinder. This procedure was adopted so that the volumes could be determined again at the conclusion of the experiment and the increase in volume used as a measure of treatment differences.

In general, the method of treatment was the same as that employed in the earlier work. In addition to untreated controls, the treatments consisted of soaking the roots for 24 hours in solutions containing 0 (tap water only), 2.5, 5, 10, 20, 40, 80, or 160 mg. of indolebutyric acid per liter of water. A surplus lot of loblolly pine was utilized by soaking the roots of similar groups for 6 hours in all concentrations except 5, 20, and 80 mg./l.

The planting was done May 5, 1944, in four well-drained beds (6 × 6 feet) of a 50-50 mixture of sand and partly

rotted sawdust. Each species was plant-
ed in two blocks, the treatments being
randomly arranged in rows of twenty
seedlings each. Thus, each row consisted
of two groups of ten seedlings on which
root volumes had been determined.
Spacing within the row was $3\frac{1}{2}$ inches
and spacing between rows was 4 inches
for loblolly and $5\frac{1}{2}$ inches for oak. A
complete nutrient solution at the rate of
$\frac{1}{2}$ oz. of salts per square foot was applied
several times during the summer.

In October, 1944, the seedlings were
removed from the beds by washing the
sand and sawdust from the roots with a
garden hose. The surviving seedlings of
the original groups of ten were tied to-
gether and the root volumes again de-
termined by water displacement.

RESULTS.—The data on survival and
root growth for red oak are presented in
table 1. A moderate trend is shown to-
ward increased survival through the
lower concentrations and up to 20 mg./l.,
with a decided drop in survival above
that concentration. The only seedlings
surviving significantly better than con-
trols were those soaked in 5 mg./l. In
contrast, survival of seedlings treated
with the highest two concentrations
was significantly poorer than controls.

Root growth was stimulated by even
the lowest concentration of acid, and a
marked increase in volume was produced
by all concentrations over 20 mg./l. The
uniformly greater root volume resulting
from all treatments between 2.5 and 20
mg./l. was not statistically significant,
although from the uniformity of the data
in this concentration range it appears
that the differences are real. The in-
crease in volume from treatments above
20 mg./l. is highly significant.

If there had been only a slight in-
crease in root growth with increasing
concentration of indolebutyric acid, the

reliability of the data representing mean
growth might be questioned, inasmuch
as the figures given are differences be-
tween mean root volumes of all seedlings
as planted and mean root volumes of
surviving seedlings at the end of the
growing season. If only small trees had
died among treated groups, these groups
would, without benefit of treatment
effects, tend to show greater mean

TABLE 1

SURVIVAL AND ROOT GROWTH OF GROUPS OF
FORTY EASTERN RED-OAK SEEDLINGS ROOT-
SOAKED 24 HOURS IN AQUEOUS SOLUTIONS OF
INDOLEBUTYRIC ACID. TREATED MAY 4,
1944; RECORD OBTAINED OCTOBER, 1944

Concentra- tion of indolebutyric acid (mg./l.)	No. surviv- ing*	Original mean root volume (ml.)	Final mean root volume (ml.)	Mean root growth (ml.)†
Untreated..	33	7.50	8.48	0.98
0.0......	35	6.88	7.43	0.55
2.5......	33	7.12	9.54	2.42
5.0......	40	7.38	10.12	2.74
10.0......	37	7.12	9.46	2.34
20.0......	36	7.62	10.00	2.38
40.0......	30	7.62	12.17	4.55
80.0......	20	7.50	15.25	7.75
160.0......	9	7.12	13.33	6.21

* Least significant difference at 5% level, 6.9; at 1% lev-
el, 9.4.
† Least significant difference at 5% level, 2.50; at 1% lev-
el, 3.37.

growth than controls. However, the
stimulation of root growth was readily
apparent (fig. 3) and the range of original
seedling sizes was not sufficient, under
any condition of differential mortality,
to cause such large treatment differences.
And, finally, if differential death of small
seedlings in treated groups had caused
the increased mean root volume, one
would expect the treatment at 5 mg./l.,
with no mortality, to have a smaller
mean growth than the treatments at
2.5, 10, or 20 mg. Such was not the case,
and therefore the conclusion seems fully

warranted that the treatment differences are real.

The results with loblolly pine are presented in table 2. The trends observed in the data for oak are not apparent in the results for pine. Mortality increased with higher concentrations of indolebutyric acid but the effect was not regular. Root growth was stimulated by most concentrations, but again there was no consistent correlation with treatment.
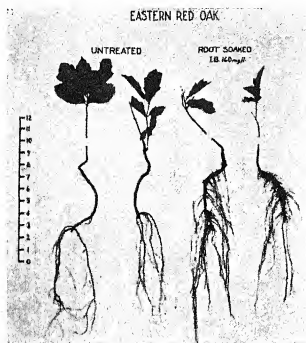
Fig. 3.—Root stimulation on 1-year-old eastern red-oak root soaked 24 hours in 160 mg. indolebutyric acid per liter of water. Survival of seedlings at this concentration greatly reduced. Photographed 5 months after planting in sand-sawdust bed.

The increase in mortality with the stronger treatments, however, indicates that for this species the series of concentrations used extended well into the toxic range.

Discussion

Soaking the roots of red oak in solutions of indolebutyric acid stimulated root growth over the entire range in concentration. This agrees with the results of others who have worked with hardwoods, as well as with some of the results obtained with hardwoods in preliminary studies at this station. The failure of tuliptree to respond with increased root growth in the preliminary studies is perhaps an exception among hardwoods.

TABLE 2

SURVIVAL AND ROOT GROWTH OF GROUPS OF FORTY LOBLOLLY-PINE SEEDLINGS ROOT-SOAKED 6 OR 24 HOURS IN AQUEOUS SOLUTIONS OF INDOLEBUTYRIC ACID. TREATED MAY 4, 1944; RECORD OBTAINED OCTOBER, 1944

Concentration of indolebutyric acid (mg./l.)	No. surviving	Original mean root volume (ml.)	Final mean root volume (ml.)	Mean root growth (ml.)
24 hours:				
Untreated..	17	0.91	3.50	2.59
0.0......	26	0.91	3.54	2.63
2.5......	19	0.85	3.32	2.47
5.0......	25	0.85	3.90	3.05
10.0......	20	0.85	4.10	3.25
20.0......	11	0.85	5.95	5.10
40.0......	15	0.84	3.93	3.09
80.0......	14	0.88	3.87	2.99
160.0......	7	0.86	4.21	3.35
6 hours:				
Untreated..	30	0.85	3.15	2.30
0.0......	24	0.85	5.04	4.19
2.5......	19	0.86	3.68	2.82
10.0......	28	0.90	4.14	3.24
40.0......	21	0.90	5.12	4.22
160.0......	10	0.88	4.40	3.52

The survival results obtained with red oak are of importance because as yet there have been no wholly satisfactory reports on survival of forest seedlings after treatment with growth regulators. Most studies have not considered this factor; some have been too small to be of much value. ROMBERG and SMITH (9, 11) found that survival was higher in treated groups, but they did not use the soaking technique. GOSSARD (5), however, was unable to find significant differences while using the same technique and species. The 1943 preliminary study at

this station showed definite increases in mortality of tuliptree following treatment with growth regulators. This effect has now been duplicated on red oak for all concentrations above 20 mg./l. Below 20 mg. there appears to be some hope of increasing survival. Soaking treatments with indolebutyric acid apparently stimulate root development of some hardwoods to a limited extent without increasing mortality, but substantial increases in development are obtained only at the expense of reduced survival.

The root response of loblolly pine was likewise in tenor with most published results. Of those working with *Pinus* or *Picea*, only PLANK (8) reported truly positive results. His work was not extensive, however, and has not been followed with confirming reports. FOWELLS (4) reported several isolated examples of stimulation but felt his results were not encouraging. In the 1942 preliminary tests, loblolly and shortleaf pine responded with increased root growth, but in later trials with loblolly these results could not be duplicated. Different lots of the same species evidently exhibit a wide range in response to growth-regulating chemicals. On the whole, it seems likely that under certain conditions soaking with growth regulators will stimulate root development on conifers, but those conditions have not yet been defined.

The mortality response of loblolly pine confirmed both previous studies at this station, as well as the work of ZACH *et al.* (14). No other work on the mortality of pines has appeared. On the basis of present evidence, however, it seems improbable that survival of pines can be increased by soaking the roots with growth regulators.

Summary

1. Seedlings of eastern red oak and loblolly pine were treated immediately before planting by soaking the roots for 24 hours (in addition to a 6-hour series for loblolly pine) in aqueous solutions of indolebutyric acid ranging in concentration from 2.5 to 160 mg. per liter. Root volumes were measured before planting and again when the seedlings were lifted at the end of the first growing season.

2. Consistent increases in root volume of red oak followed soaking in all concentrations of indolebutyric acid, and statistically significant increases occurred with concentrations of 40 mg./l. or more.

3. A barely significant increase in survival followed treatment of red oak with 5 mg./l. On the other hand, a marked and highly significant increase in mortality followed treatment with 80 mg./l. or more. The largest increases in root volume of red oak were associated with the largest increases in mortality.

4. Treatments generally increased the root volume of loblolly pine, although there was no consistent relationship between root volume and concentration.

5. A marked increase in mortality of loblolly pine followed treatment with concentrations above 20 mg./l.

SOUTHERN FOREST EXPERIMENT STATION
    GULFPORT, MISSISSIPPI

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EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE GROWTH OF GRASS PLANTS

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Introduction

The use of 2,4-dichlorophenoxyacetic acid as a selective herbicide for eradicating such turf weeds as dandelion and plantain has recently been suggested (4, 5). Most of the experimental results on this method of control indicate that application of the acid or salts (sodium or ammonium) of the acid, at the rate of about 1½ pounds per acre, does not noticeably affect the appearance of well-established bluegrass or some other varieties of grass commonly used in lawns. Attention for the most part has been directed toward a study of the weed-killing properties of 2,4-dichlorophenoxyacetic acid, rather than toward its effects on the germination and growth of young grass seedlings or older grass plants (1, 2, 3).

The present experiments, in which the treatments were applied to the soil immediately following planting, were undertaken for the purpose of determining the effect of the acid on (a) the emergence of grass seedlings of different species, (b) the subsequent growth of the various grasses, and (c) grass plants well established when sprayed.

Experimental data

SEEDLING EMERGENCE

METHODS.—Weighed amounts of seeds of redtop (*Agrostis alba*), creeping red fescue (*Festuca rubra*), and Kentucky bluegrass (*Poa pratensis*) were planted in composted soil contained in clay pots 12 inches in diameter and 6 inches deep, with bottom drainage. On October 20,

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1944, forty pots were sown with each species of grass seed at the rate of 0.5 gm. per pot, a total of 120 pots being used.

After the seeds had been covered lightly with soil, the surface was sprayed evenly with a measured amount of an aqueous mixture of the acid, applied with a hand sprayer of one-pint capacity operating at a pressure of 80 pounds. The aqueous mixture contained 0.1% of the acid and 0.5% Carbowax 1500 applied at

TABLE 1

AVERAGE PERCENTAGE EMERGENCE OF GRASS SEEDLINGS 1 WEEK AFTER SPRAYING SOIL SURFACE WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500; SPRAY APPLIED IMMEDIATELY AFTER SEEDS WERE PLANTED. AVERAGE NUMBER OF SEEDLINGS THAT EMERGED IN UNSPRAYED PLOTS DESIGNATED AS 100%

Rate of treatment (equivalent lb. per acre)	Redtop	Fescue	Bluegrass
0	100	100	100
$\frac{3}{4}$	72	110	114
$1\frac{1}{2}$	17	102	80
$2\frac{1}{4}$	17	90	71
3	5	83	68

rates equivalent to 2, 4, 6, and 8 gallons per thousand square feet—or $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. Similar pots of soil were planted but left unsprayed as controls. The treatments were then arranged in randomized blocks on a greenhouse bench so that two pots of the same variety and treatment constituted a plot. There were four replications of each plot. Temperature was maintained at $65°$–$75°$ F. throughout the experiment.

The number of grass seedlings that appeared above the surface of the soil 7 days after planting was recorded and designated as emergence. Although some

seedlings may have emerged after this time, no subsequent counts were made owing to the difficulty involved in obtaining an accurate estimation of their number.

RESULTS.—Spray mixture at a rate equivalent to $2\frac{1}{4}$ and 3 pounds of acid per acre reduced the emergence of all three varieties of grass seedlings as compared with the number that appeared above the surface of the unsprayed soil (table 1). The emergence of plants of redtop was much more drastically affected by the chemical than was that of either fescue or bluegrass. In the case of fescue, a greater percentage of seedlings appeared above the surface of soil sprayed with small amounts of the acid ($\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre) than emerged from the unsprayed plots. A greater number of bluegrass seedlings also appeared above the surface of soil sprayed with the mixture at a rate equivalent to $\frac{3}{4}$ pounds per acre than emerged from unsprayed soil. This experiment was continued so as to determine subsequent effects of treatment on seedling growth.

SEEDLING GROWTH

METHODS.—After the emergence counts had been taken and the leaves of grass had attained a length of approximately 3 inches, they were cut back uniformly to a height of 2 inches, and this height was maintained by clipping at weekly or bi-weekly intervals. The fresh weights of the clippings were recorded.

At the end of 5 weeks after treatment, all plants in one of the pots in each replicate of each treatment were pulled up, and each of these pots was then reseeded with 0.5 gm. of seed of the respective varieties. The weights of weekly clippings from plants that developed in these reseeded plots were recorded and are summarized separately.

RESULTS.—The seedlings that emerged in the treated soil did not outwardly appear to differ from those in the untreated soil. The weights of clippings from all treated plots of the original seeding of redtop were significantly less than top growing in soil sprayed at the rate of $1\frac{1}{2}$ pounds per acre were not appreciably less than those of the unsprayed plots. The production of clippings by redtop in plots sprayed at the rates of $2\frac{1}{4}$ and 3 pounds per acre was appreciably less

TABLE 2

AVERAGE FRESH WEIGHT IN GRAMS OF GRASS CLIPPINGS COLLECTED AT WEEKLY INTERVALS AFTER PLANTS HAD GERMINATED IN SOIL SPRAYED WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500

RATE OF TREATMENT (EQUIVALENT LB. PER ACRE)	WEEKS AFTER TREATMENT												TOTAL
	3	4	5	6	7	8	9	10	11	12	13	14	
	Redtop												
0	3.7	9.0	8.6	4.6	5.4	5.5	6.6	3.8	2.1	2.4	2.1	5.2	59.0
$\frac{3}{4}$	2.0	5.7	10.1	4.2	5.9	5.6	4.0	1.9	2.7	2.0	6.4	56.6	
$1\frac{1}{2}$	0.1	0.3	1.8	1.3	2.5	3.3	4.0	2.4	2.0	2.8	2.7	7.6	30.8
$2\frac{1}{4}$	0.0	0.1	0.6	0.6	0.9	1.3	1.9	1.4	1.4	2.5	1.5	4.4	16.6
3	0.0	0.0	0.2	0.2	0.4	0.6	0.7	0.8	0.9	1.2	1.0	4.2	10.2
	Fescue												
0	3.0	2.3	3.4	2.4	3.0	3.1	4.1	3.4	2.4	2.5	2.6	5.1	37.3
$\frac{3}{4}$	1.3	1.0	2.2	1.7	1.4	2.6	3.2	2.6	1.7	2.1	2.0	4.4	26.2
$1\frac{1}{2}$	0.6	0.6	1.6	0.8	1.5	1.7	1.9	1.6	1.4	1.5	1.9	3.1	18.2
$2\frac{1}{4}$	0.4	0.4	1.0	0.6	1.0	1.1	1.2	1.3	1.1	1.7	1.6	3.7	15.1
3	0.5	0.4	0.9	0.7	1.1	1.5	1.5	1.2	1.1	1.1	1.3	3.4	15.3
	Bluegrass												
0	1.6	4.2	6.0	3.2	3.5	3.6	4.4	3.4	2.3	2.8	2.8	5.8	43.6
$\frac{3}{4}$	0.1	0.7	3.2	1.9	3.3	3.5	3.8	3.0	2.0	2.6	2.3	4.3	30.7
$1\frac{1}{2}$	0.0	0.3	2.2	1.6	2.6	2.7	2.6	2.0	1.6	2.5	2.1	4.0	24.2
$2\frac{1}{4}$	0.0	0.2	1.9	1.2	2.1	2.5	2.1	1.7	1.4	1.9	1.7	3.8	20.5
3	0.0	0.1	1.1	0.9	1.5	1.8	1.7	1.3	1.2	1.4	1.6	3.5	16.1

those from the unsprayed ones for a period of 1 month (table 2). During this period, however, grass growing in soil sprayed at a rate equivalent to $\frac{3}{4}$ pound of the acid per acre multiplied, apparently by means of stolons or rhizomes, and during the last 10 weeks of the experiment it was as productive as was that growing in untreated soil. At the end of 9 weeks, the weights of clippings of redtop growing in soil sprayed at the rate of than that of the unsprayed plots during the entire period of 14 weeks, at which time the experiment was discontinued.

Fescue growing in soil sprayed at the rate of $\frac{3}{4}$ pound of the acid per acre became increasingly productive, and after the fifth week the weights of clippings obtained were not appreciably less than those from the unsprayed plots. Plots of fescue sprayed at rates of $1\frac{1}{2}$ pounds or

more per acre failed to equal the unsprayed plots in productivity.

The productivity of bluegrass plots was significantly reduced on the basis of the clippings obtained, but within 7

failed to recover during the 14-week period.

Five weeks after application of the spray treatments there were no apparent or measurable effects, either on the

TABLE 3

AVERAGE FRESH WEIGHT IN GRAMS OF CLIPPINGS FROM GREENHOUSE GRASS PLOTS RESEEDED 5 WEEKS AFTER SOIL SURFACE WAS SPRAYED WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX. PLANTS OF FIRST SEEDING REMOVED BEFORE RESEEDING

Rate of treatment (equivalent lb. per acre)	Weeks after seeding								Total
	3	4	5	6	7	8	9	11	
Redtop									
0	1.0	4.3	2.3	2.3	3.0	3.3	7.7	12.1	36.0
¾	1.7	4.8	3.2	2.1	3.0	3.4	8.0	11.5	37.7
1½	2.4	5.5	3.8	3.3	3.2	2.9	9.7	16.1	46.9
2¼	2.0	5.1	3.6	3.0	3.0	2.5	6.7	10.7	36.6
3	2.0	4.6	3.1	2.6	2.4	2.9	9.8	15.3	42.7
Fescue									
0	1.4	1.0	0.5	0.6	0.8	0.8	2.8	10.5	18.4
¾	1.7	1.1	0.6	0.5	0.8	0.9	3.4	9.1	18.1
1½	2.2	1.0	0.9	0.6	0.9	0.7	2.6	7.5	16.4
2¼	2.1	1.2	0.8	0.7	0.7	1.0	4.7	9.0	20.3
3	2.4	1.4	0.9	0.7	0.9	1.1	4.1	9.6	21.1
Bluegrass									
0	0.4	1.2	1.0	1.1	1.6	2.0	3.7	6.6	17.6
¾	0.3	1.1	1.3	1.3	2.0	2.5	5.0	7.0	20.5
1½	0.3	1.4	1.7	1.6	2.2	2.4	4.7	8.1	22.4
2¼	0.5	1.5	1.4	1.5	1.6	1.9	6.2	8.7	23.3
3	0.4	1.5	1.5	1.7	1.8	2.3	4.8	7.6	21.6

weeks after seeding plants growing in soil sprayed at the rate of ¾ pound of acid per acre attained a production of clippings not significantly less than that of the unsprayed plots. Eleven weeks were required, however, before plots treated at the rates of 1½ and 2¼ pounds per acre were as productive as were the unsprayed ones. Bluegrass growing in soil sprayed at the rate of 3 pounds per acre

emergence of seedlings in reseeded plots or on the subsequent growth of the grass, indicating that the acid had been either inactivated or leached from the soil during this time (table 3).

GROWTH OF BENTGRASS

METHODS.—On October 23, 1944, nine clumps of stolons of the Washington strain of creeping bentgrass (*Agrostis*

palustris), each consisting of six stolons, selected for size and uniformity, were planted 1–2 inches apart in each of forty clay pots containing composted soil. The pots were similar to those already described. On November 11, after the grass had become well established, the pots were separated into five groups of eight pots each. The grass in each of four groups was then sprayed with an aqueous mixture containing 0.1% of the acid and 0.5% Carbowax 1500 at a rate equiva-

TABLE 4

AVERAGE FRESH WEIGHT IN GRAMS OF BENT-GRASS CLIPPINGS PRODUCED PER POT IN GREENHOUSE, COLLECTED AT MONTHLY INTERVALS, AFTER SPRAYING WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLORO-PHENOXYACETIC ACID AND 0.5% CARBOWAX 1500

RATE OF TREATMENT (EQUIVALENT LB. PER ACRE)	MONTHS AFTER TREATMENT				TOTAL
	1	2	3	4	
0	18.4	16.2	14.1	32.7	81.4
$\frac{3}{4}$	14.4	13.1	16.9	32.7	77.1
$1\frac{1}{2}$	9.1	8.2	11.3	30.5	59.1
$2\frac{1}{4}$	8.7	7.8	8.8	29.3	54.6
3	6.5	5.9	7.3	26.4	46.1

lent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, or 3 pounds per acre, respectively. The eight remaining pots were left untreated. After treatment, the pots were arranged in randomized blocks on a greenhouse bench with four replications of each treatment. Throughout the experiment the grass was clipped back to a height of 2 inches at approximately weekly intervals and the fresh weights of the clippings recorded.

RESULTS.—The total weight of clippings produced by bentgrass was reduced significantly below that of the unsprayed plants as the result of treatment at all rates of the spray mixture used (table 4). Within 1 month following treatment the sprayed plants developed enlarged re-

gions at the nodes of the rhizomes, and the leaf blades were a less intense green and somewhat narrower than were those on the unsprayed plants. The sprayed plants gradually improved in appearance, however, and at the end of the experiment there were no apparent differences between them and the controls.

The growth of bentgrass was not significantly affected by the spray mixture during the first week following treatment. All treatments depressed growth during the second, third, and fourth weeks, so that the total weight of clippings during the first month was significantly less than in the case of unsprayed plants (table 4). Plots sprayed at the rate of $\frac{3}{4}$ pound per acre recovered, and following the first month after treatment they produced an amount of clippings equal to or slightly greater than that from the unsprayed plots. Cuttings from plots sprayed at the rates of $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre weighed 6.7, 10.4, and 19.3% less, respectively, than those cut from unsprayed plots during the fourth month after treatment.

ESTABLISHED TURF

METHODS.—On March 26, 1945, an area of turf having a uniformly mixed stand of the three species of grass—redtop, creeping red fescue, and Kentucky bluegrass—was selected and marked off into thirty parallel strips 2 feet wide and 25 feet long. Treatments consisted in spraying the individual strips with a water mixture containing 0.1% of the acid and 0.5% Carbowax 1500 at the same equivalent rates as were used in the previous greenhouse experiments—0, $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre. The various treatments were assigned at random to the plots so as to make six randomized blocks.

A hand-pressure knapsack sprayer was

used in applying the treatment, and plyboard shields were held along the sides of each area as it was being sprayed to avoid drift of the mixture. All weeds on the entire area were removed by hand at the beginning of the experiment to eliminate competition between the weeds and grass and to make it possible to observe the direct effect of the acid on growth of the grass.

At intervals of several weeks, a strip 18 inches in width was mowed lengthwise through the middle of each plot, the

following this period there was no consistent difference between these two treatments. The total weight of clippings obtained from plots sprayed with an equivalent of $\frac{3}{4}$ pound per acre over a 6-month period was not appreciably less than of those from the unsprayed plots.

During the 6-month period following treatment, the total weight of clippings from plots sprayed at the rate of $1\frac{1}{2}$ pounds per acre was 11.6% less than that produced by the unsprayed plots. The growth of grass on plots sprayed with

TABLE 5

Average fresh weight in grams of clippings from grass turf composed of Kentucky bluegrass, creeping red fescue, and redtop, compared with that for areas of equal size sprayed with aqueous mixture containing 0.1% 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax

Rate of treatment (equivalent lb. per acre)	Days after treatment									Total
	18	37	52	78	88	102	126	151	188	
0	510	445	458	424	396	482	423	877	269	4284
$\frac{3}{4}$	381	506	525	475	328	457	353	842	274	4141
$1\frac{1}{2}$	349	420	478	370	311	417	351	823	268	3787
$2\frac{1}{4}$	339	390	507	470	305	425	348	777	275	3836
3	344	352	479	432	340	465	373	779	267	3831

mower being equipped with a grass catcher. The weights of the clippings were recorded immediately after cutting. The entire area was mowed evenly after the clipping samples had been taken.

RESULTS.—Grass in the treated plots showed no apparent signs of injury, but weights of clippings taken 18 days following treatment indicated that the growth of plants treated with even the lowest spray concentration (equivalent to $\frac{3}{4}$ pound per acre) was significantly less than that of the untreated plants (table 5). During the second month after treatment, clippings from plots sprayed with an equivalent of $\frac{3}{4}$ pound per acre weighed approximately 14.6% more than did those from the unsprayed plots; but

greater amounts of the acid ($2\frac{1}{4}$ and 3 pounds per acre) was not consistently different throughout the experiment from growth on those sprayed with $1\frac{1}{2}$ pounds per acre. In all cases the treatment caused an initial depression in the rate of growth. This depression gradually disappeared, and at the end of approximately 2 months there was no significant difference between the weights of clippings from the treated and those from the untreated plots.

During the latter part of the 6-month period following treatment, weed seedlings grew in the control plots but not in any of the treated ones. In September, 6 months after treatment, the grass and weed leaves in samples of clippings from

the unsprayed plots were separated. The weights of the weed clippings in six replicated unsprayed plots were 32, 34, 32, 30, 31, and 30% of the total weight of clippings from each plot, respectively, while the weight of weed leaves in clippings from all other treatments was estimated to be only a fraction of 1% of the total weight of the samples, since only a few weed leaves were found in some of the samples of grass taken from the treated plots, while others were completely free of weeds.

Discussion

In previous experiments (3, 5), satisfactory stands of Kentucky bluegrass seedlings were established in lawn areas by broadcasting the seeds just prior to spraying with a water mixture of 2,4-dichlorophenoxyacetic acid in the fall. These earlier results indicated that at least some of the seeds sown on a well-established lawn at the time of spraying may germinate and grow. The number which become established, however, may depend somewhat on protection from the spray mixture that is afforded by the seeds and soil by leaves of grass and weeds present on the area at the time of treatment. Results of the present experiments indicate that it is somewhat more difficult to establish seedling grass plants in bare soil that has been sprayed than it is in unsprayed soil.

The detrimental effects of the spray mixtures on soil were not persistent under greenhouse conditions, however, since seeds of the three species of grass germinated readily and became established when planted in the soil 5 weeks after its surface had been sprayed. In experiments reported by others (2), seeds of several crop plants sown in soil 58 days

after it was treated with 2,4-dichlorophenoxyacetic acid germinated readily and the plants grew vigorously. Also, seeds of Kentucky bluegrass became established in a lawn area that had been treated with the acid 54 days earlier.

Effort should be made to avoid spraying the acid mixture directly onto fallow soil in which grass seedlings are to be established soon after treatment. On the basis of the present experiments it would appear that a satisfactory stand of new grass can be more readily obtained if the seeds are planted 4–6 weeks after treatment, rather than if planted at the time the spray treatments are applied.

The species of bentgrass used responded somewhat differently from bluegrass, fescue, and redtop when the leaves were sprayed with the mixture, in that it showed outward signs of the detrimental effect of the acid and its growth was markedly suppressed, while other grasses (bluegrass, fescue, and redtop) did not change appreciably in appearance as the result of treatment, and their growth was only temporarily inhibited. Of course, the results obtained under the conditions of these experiments might vary, depending upon the strains of grasses used, the season of the year, and the weather conditions under which the plants were grown. It should also be mentioned that the effect on the growth of the grass owing to the elimination of weed competition through the herbicidal effects of the treatment has not been studied, since all weeds were removed from both treated and untreated areas at the beginning of the experiments.

The observed depression of growth of grasses as a result of application of the acid to their leaves would appear to be of little practical consequence, in so far as

grass plants not used as forage are concerned. Further experiments are necessary to determine the significance of the effect of the acid on the productivity of pastures or range areas where the chemical might be used for herbicidal purposes. The results of these experiments, however, indicate that the depressing effects on relatively mature grass are not lasting; and where weeds in pastures or range areas seriously reduce the amount of grass produced, it would appear feasible to eliminate them by the use of 2,4-dichlorophenoxyacetic acid.

Summary

1. Potted soil in which Kentucky bluegrass, redtop, and creeping red fescue grass seeds had been planted was sprayed with a water mixture containing 0.1% 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. The treatments reduced the number of redtop seedlings that appeared in 28, 83, 83, and 95%, respectively. A slightly greater number of fescue seedlings emerged from the soil sprayed at rates equivalent to $\frac{3}{4}$ and $1\frac{1}{2}$ pounds of acid per acre, while the heavier applications reduced emergence by 10 and 17%, in comparison with the unsprayed soil. Fourteen per cent more bluegrass seedlings appeared in the soil sprayed at a rate equivalent to $\frac{3}{4}$ of a pound per acre than emerged in unsprayed soil. The heavier applications reduced the emergence of bluegrass by as much as 32% below that of untreated soil.

2. Grass seedlings that became established in soil sprayed with various amounts of the water mixture of the acid were allowed to grow under greenhouse conditions for a period of 14 weeks, and their rate of growth was measured each week by means of weights of leaf clippings. Weights of clippings from plants of all three species in treated soil were less than those from the same species grown in untreated soil. Each of the three species, however, showed a tendency to recover from the effects of the sprays. Judging by weights of clippings, the grass seedlings in soil treated with $\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre had recovered after 5–11 weeks, but at the heavier rates ($2\frac{1}{4}$ and 3 pounds) per acre the grass was still retarded in growth after 14 weeks.

3. The detrimental effects of the acid in the soil were only temporary, since redtop, fescue, and bluegrass seeds germinated and the seedlings became established in treated soil as readily as in untreated soil, when the seeds were planted 5 weeks after the soil was sprayed.

4. The growth of well-established creeping bentgrass was depressed by spraying with the water mixture at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. Plants sprayed at rates equivalent to $\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre recovered during a period of 3–4 months following treatment.

5. The appearance of turf growing out-of-doors and made up of Kentucky bluegrass, creeping red fescue, and redtop was unchanged after application as a water spray at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre. Weight of clippings was reduced, however, during a period of 1–2 months after treatment, but after this interval there was no difference between the growth of sprayed and unsprayed plots.

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EFFECT OF GROWTH-REGULATING SUBSTANCES ON
THE DEVELOPMENT OF APPLE SCALD

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Introduction

During an investigation by C. L. HAMNER and the senior writer concerning the effects of growth-regulating substances on the keeping quality of apples in cold storage, it was observed that the treated fruits developed considerably less scald than the untreated ones during the storage season of 1943-44. The growth substances had been applied to the fruits after harvest and just prior to placing in cold storage. The effects of the treatments were significant enough to warrant further investigation in the storage season of 1944-45. The results reported here for these two seasons are considered preliminary, but they show a positive effect of treatment and are in sufficient agreement to warrant a report at this time.

Methods

Apple varieties used in the investigation for the 1943-44 season were Grimes Golden, Stayman Winesap, and York Imperial. In the 1944-45 season the first two varieties were used, and Arkansas (Mammoth Black Twig), a variety especially susceptible to scald, was substituted for York Imperial.

In treating the fruits, duplicate lots of approximately 1 bushel selected at random from the entire bulk of material were used for each treatment. The treatments were applied by two methods: (a) as a dip, which was a lanolin emulsion containing varying amounts of the different growth substances; and (b) as an aerosol, applied by the use of a liquefied gas containing the chemical in solution (5).

The lanolin emulsion for the dip treatments was prepared in lots of 10 liters each and contained 4 gm. of pure lanolin per liter. In preparing the emulsion, 7.5 gm. of stearic acid was melted in 100 ml. hot water and 2.7 gm. of trimethanolamine added while stirring. Forty grams of previously melted lanolin was then added and the mixture stirred vigorously until cool. Water was added to the thick emulsion, slowly at first, with constant stirring, until the 10 liters were obtained. In preparing the final mixtures for dipping, the growth substances were first dissolved in 95% ethyl alcohol in the proportion of 1:10, and the necessary quantities of the alcoholic solution were added to the prepared lanolin emulsion to give the desired concentrations. Each growth

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substance (or a mixture of them) was applied in one or all of three concentrations, the total amount of the substances present being 10, 100, and 500 p.p.m., respectively. Following treatment, the fruits were allowed to dry and were placed in boxes in 31° F. storage for a period of 4–5 months.

The growth substances used were α-naphthaleneacetic acid, β-indolebutyric acid, and a mixture in equal parts of these two acids, plus β-naphthoxyacetic acid and α-naphthalene acetamide.

For the aerosols, dimethyl ether (b.p. −27° C.) was used as the volatile solvent. In each instance the aerosol mixture consisted of 1% of the growth substance, 10% of cyclohexanone as solvent, and 89% of dimethyl ether as the volatile agent. The fruits to be treated were spread on a drying screen and the aerosol was directed on them from above and below to insure complete coverage.

In classifying the scalded fruits, three arbitrary degrees of severity of scald were designated as slight, medium, or severe. Slight scald denoted discoloration of a mild intensity, or that a very small area was affected on the individual fruits, the disorder being considered so slight as to be unobjectionable. Medium scald denoted moderate discoloration, such that the fruits would probably be discriminated against on the market. Severe scald indicated fruits that were decidedly objectionable, the scalded area including 50% or more of the fruit surface. Figure 1 illustrates three lots of fruits sorted into four grades (sound, and the three degrees of scald).

Results

ARKANSAS (MAMMOTH BLACK TWIG) EXPERIMENTS, 1944–45

During the 1944–45 season, the most striking results were obtained with the Arkansas variety. The fruit was harvested October 19, 1944, and placed at 31° F. for 5 days until treated on October 24. On April 18, 1945, the fruits were removed from cold storage and placed at 70° F. for 7 days to allow time for maximum scald development. Final examination for scald was made on April 25. Considerably less scald was found in all lots treated with the compounds than in the check lots (table 1). On the basis of scald development, the best treatment consisted of the lanolin emulsion containing 100 p.p.m. α-naphthaleneacetic acid. Fruits receiving this treatment were 33.1% sound and 33.7% slightly scalded, as compared with the check (untreated) fruits in which only 9.1% were sound and 15.6% slightly scalded. Medium and severe scald in these same lots were, respectively, 22.3 and 9.0% in the treated fruits and 19.9 and 49.9% in the untreated. Little difference was found in the percentages of sound and slightly scalded fruits among the different treated lots. The lanolin emulsion without growth regulators also showed a tendency to reduce scald development, as indicated in table 1 and figure 1.

From these results it may be seen that the intensity of scald development, as well as the total number of fruits affected by the disorder, was reduced by treatment with growth-regulating substances.

STAYMAN WINESAP EXPERIMENTS 1943–44 AND 1944–45

Although a marked reduction in scald development was obtained on Stayman Winesap apples in both storage seasons, the degree of scald reduction differed considerably. The variance in response may have been due to differences in maturity of the fruits when picked, to different sources of the material, or to delay in treating the fruits after harvest (1–3).
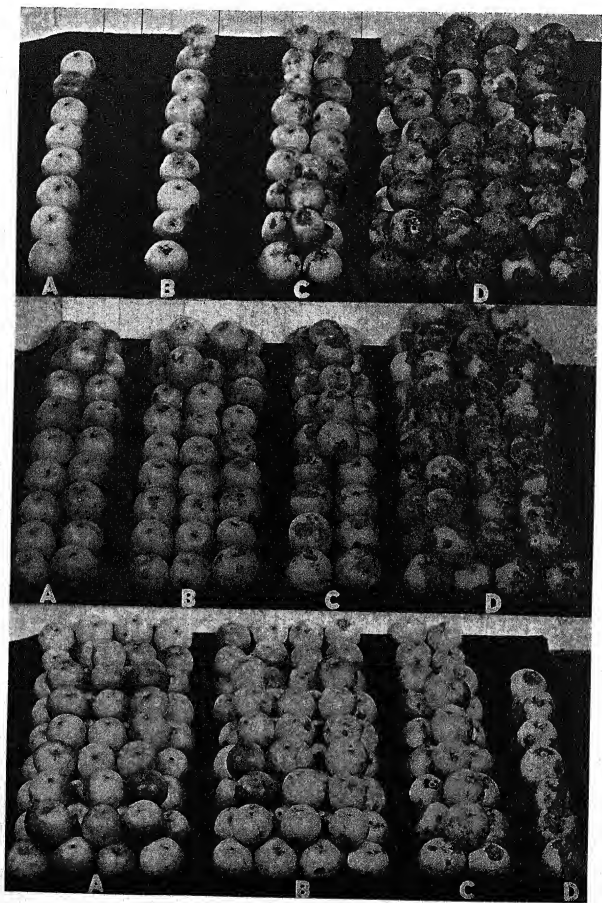
FIG. 1.—Scald development on Arkansas apples at end of storage period on April 25, 1945. A, sound; B, slightly scalded; C, moderately scalded; D, severely scalded. Upper row, untreated fruits. Middle row, fruits dipped in lanolin emulsion. Lower row, fruits dipped in lanolin emulsion containing 500 p.p.m. α-naphthaleneacetic acid.

Fruit for the 1943–44 storage season was picked on October 10, 1943, from an orchard near Mt. Jackson, Virginia. It was held for 5 days at 31° F. prior to treatment on October 15. Following treatment, the apples were held in 31° F. storage until April 21, when they were removed to 70° F. and held for 7 days before final examination on April 28. It was on the treated fruits of this lot that the first observations were made of strik-

17.2, respectively; in contrast, comparable untreated fruits were 62.3 scalded. The percentages of sound fruits for the same treatments were 70.7, 67.5, and 75.4, respectively, whereas the control fruits were 31.1% sound.

In the 1944–45 season the fruit was harvested on the Plant Industry Station grounds at Beltsville, Maryland, on October 8, 1944, and stored at 31° F. for 16 days prior to treatment on October

TABLE 1

EFFECT OF GROWTH-REGULATING SUBSTANCES ON SCALD DEVELOPMENT ON ARKANSAS (MAMMOTH BLACK TWIG) APPLES, 1944–45. FRUITS TREATED AND STORED AT 31° F. ON OCTOBER 24, 1944; REMOVED FROM COLD STORAGE ON APRIL 18, 1945, AND PLACED AT 70° F. FOR 7 DAYS PRIOR TO MAKING FINAL SCALD RECORDS

TREATMENT	SOUND FRUIT (%)	PERCENTAGE SCALD			TOTAL DECAY (%)
		Slight	Medium	Severe	
Naphthaleneacetic acid (in lanolin emulsion)					
10 p.p.m.	24.6	37.7	17.5	11.6	8.2
100 p.p.m.	33.1	33.7	22.3	9.0	2.0
500 p.p.m.	30.3	33.3	16.8	16.8	3.3
1% aerosol	21.3	34.3	29.7	10.5	4.2
Mixture*					
100 p.p.m. in lanolin emulsion	31.3	34.3	17.5	14.2	2.7
1% aerosol	27.5	35.4	19.9	15.2	2.0
Lanolin emulsion only	16.0	21.5	19.5	40.4	2.6
Check (untreated)	9.5	15.6	19.9	49.9	5.1

* Equal parts of naphthaleneacetic acid, indolebutyric acid, naphthoxyacetic acid, and naphthalene acetamide, in lanolin emulsion or in aerosol.

ing scald reduction due to the compounds. In these preliminary investigations three concentrations (10, 100, and 500 p.p.m.) in lanolin emulsion of naphthaleneacetic acid, of indolebutyric acid, and of the mixture of four growth substances were employed. All treatments resulted in less scald than occurred in the untreated control. Little variation in the percentage of scalded fruits, due to varying concentrations of the growth regulators, was found. The average percentages of scalded fruits of all lots treated with naphthaleneacetic acid, indolebutyric acid, and the mixture were 21.5, 25.6, and

24. Final examination of the fruits was made April 6, after they had been held for 1 week at 70° F. to allow time for maximum scald development. The greatest scald reduction was obtained with naphthaleneacetic acid at 500 p.p.m. in lanolin emulsion (table 2). The effectiveness of this substance declined with decrease in concentration. The mixture of the four growth substances in lanolin emulsion was somewhat less effective than the same concentration (100 p.p.m.) of naphthaleneacetic acid used alone, while the mixture used as aerosol produced no reduction in scald. Consider-

able injury was produced by the aerosol application, with subsequent decay of fruits, a number of which otherwise might have been classified as free from scald. Some scald reduction was produced by the lanolin emulsion alone, but incidence of decay was very high from this treatment, owing chiefly to one of the duplicate lots in which 51% of the fruits showed some decay. No explanation can be given for the high percentage in this lot.

check and a second check lot dipped in a lanolin emulsion completed this test. The apples were picked September 15, treated and placed in storage at 31° F. on September 16, and examined for scald on January 11, after 1 week at 70° F.

Although the percentages of sound (not scalded) fruits did not vary greatly between the treated and untreated lots (8.6 and 9.3%, respectively), the intensity of the scald was reduced by treatment, so that the average percentage of

TABLE 2

EFFECT OF GROWTH-REGULATING SUBSTANCES ON SCALD DEVELOPMENT ON STAYMAN WINESAP APPLES, 1944–45. FRUIT HARVESTED OCTOBER 8, 1944; STORED AT 31° F. FOR 16 DAYS; TREATED AND RE-STORED AT 31° F. OCTOBER 24; REMOVED FROM STORAGE MARCH 31, 1945; PLACED AT 70° F. FOR 7 DAYS AND EXAMINED FOR FINAL SCALD DEVELOPMENT APRIL 6

TREATMENT	SOUND FRUIT (%)	PERCENTAGE SCALD			TOTAL DECAY (%)
		Slight	Medium	Severe	
Naphthaleneacetic acid (in lanolin emulsion)					
10 p.p.m.	29.5	12.6	23.7	24.5	9.7
100 p.p.m.	35.6	11.9	20.6	14.9	17.0
500 p.p.m.	44.1	14.2	20.4	7.8	13.5
1% aerosol	29.7	13.9	21.7	13.7	21.0†
Mixture*					
100 p.p.m. in lanolin emulsion.	29.8	16.7	17.2	24.3	12.0
1% aerosol	20.4	14.7	19.6	18.6	26.7†
Lanolin emulsion only	23.1	12.7	16.3	31.9	33.7
Check (untreated)	20.5	19.8	31.0	17.5	11.2

* Equal parts of naphthaleneacetic acid, indolebutyric acid, naphthoxyacetic acid, and naphthalene acetamide, in lanolin emulsion or in aerosol.

† Includes fruits injured by aerosol spray, too much solvent having been applied.

GRIMES GOLDEN AND YORK IMPERIAL EXPERIMENTS, 1943–44 AND 1944–45

Grimes Golden apples that were treated with growth substances had consistently less scald than the untreated checks, although the reduction of scald was not so great as that found in the Arkansas and Stayman Winesap varieties. The only treatments applied to Grimes Golden during the 1943–44 season were three concentrations of the mixture of growth substances. An untreated

severely scalded fruits in the treated lots was 40.5 and in the untreated lots 61.9. Furthermore, the percentages of fruits with slight and medium scald averaged 20.9 for the treated lots and 8.6 for the untreated check. As was found with the other varieties, treatment with lanolin emulsion without growth substances tended to reduce the intensity of scald on Grimes Golden. Similar results were obtained on Grimes Golden during the 1944–45 season, the chief effect of the

treatments being to bring about a reduction in the severity of the scald.

Scald development was light on both the treated and untreated lots of the York Imperial variety for the 1943–44 season, and no attempt was made to classify according to degree of scald. The untreated check lot had the greatest percentage of scalded fruits, 13.1% showing some degree of the disorder. The average percentages of scalded fruits in the treated lots were as follows: naphthaleneacetic acid 9.7, indolebutyric acid 7.1, and the mixture 5.1. Thus, the reduction of scald, although slight, was consistent for all treatments.

The use of lanolin emulsion for application of the growth substances did not seem to affect decay development in the fruits of any variety, the total decay for the lanolin-dipped fruits in all the experiments being 10.0% as compared with 12.0% for the untreated. Injury occurred in some instances where the aerosol vapor was used, probably because an excessive amount of the cyclohexanone solvent was applied during treatment. Pathogens readily invaded these injured areas, with the result that a high percentage of decay occurred.

The lanolin emulsion alone usually effected a slight reduction in scald. Figure 1 illustrates a lot of Arkansas apples dipped in lanolin emulsion in comparison with an untreated lot and with a lot dipped in lanolin emulsion containing 500 p.p.m. naphthaleneacetic acid.

Discussion

Growth-regulating chemicals applied to apples to prevent pre-harvest drop may affect their storage quality indirectly because of the more advanced maturity of later pickings made possible by the sprays. HALLER (4), however, found that α-naphthaleneacetic acid when applied

to the trees as a pre-harvest spray had no effect on the firmness of the fruit or on the development of decay, breakdown, or scald during subsequent storage when sprayed were compared with unsprayed apples picked at the same time.

Acceleration of ripening of several kinds of fruits due to growth substances applied after harvest has been reported by MITCHELL and MARTH (6), and the effect of these substances in reducing apple scald may have been associated with their effect on the rate of ripening, since it is known that the incidence of scald is less on mature than on immature fruits (1–3).

In this investigation, less scald occurred on apples treated with growth substances than on the untreated fruit. Also, the various varieties tested responded differently, the most striking results being obtained with Arkansas. This does not necessarily mean that the growth-regulating compounds are more effective on Arkansas; a number of uncontrolled factors, such as varietal susceptibility to scald, maturity of fruit when picked, and delay before treatment, may all have contributed to the results obtained.

All the growth substances employed had a similar effect on scald, no one compound being obviously superior to another. In all probability, more effective chemicals or combinations of them for reducing scald may be found.

Although the application of these growth substances has not decreased scald development to a satisfactory degree, it has given sufficient promise to encourage further investigation of the problem.

Summary

1. Experiments were conducted on apples during the 1943–44 and 1944–45 storage seasons to determine the effect

of growth-regulating substances on development of fruit scald.

2. Two substances, α-naphthaleneacetic acid and β-indolebutyric acid, and a mixture consisting of equal parts of α-naphthaleneacetic acid, α-naphthalene acetamide, β-naphthoxyacetic acid, and β-indolebutyric acid were tested at 10, 100, and 500 p.p.m. concentration in lanolin emulsion (0.4% lanolin) on fruits of Arkansas (Mammoth Black Twig), Stayman Winesap, Grimes Golden, and York Imperial varieties. The fruits were treated after harvest and prior to 31° F. storage by dipping in lanolin emulsion containing the various growth regulators or by using a liquefied-gas aerosol of the substance. The amount of scald development was noted at the end of the storage season of each variety after the fruits had been held for 7 days at 70° F. to allow time for maximum scald development.

3. Treatment resulted in consistently less scald than on untreated fruit. The greatest reduction occurred on the Arkansas variety, where there was an average of 24% more unscalded fruits in the treated than in the untreated lots. Less marked reduction occurred on Stayman Winesap, Grimes Golden, and York Imperial.

4. No appreciable differences were noted between the various compounds or between the concentrations employed. The lanolin-emulsion dip treatments appeared to give slightly better control of scald than did the aerosol treatments.

5. The decrease in scald development was manifest as a reduction both in severity of scald and in total number of fruits affected.

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EMBRYO SAC AND FERTILIZATION IN CYPRIPEDIUM SPECTABILE

B. G. L. SWAMY

Introduction

In 1907, PACE (5) discussed the development of the embryo sac and fertilization in four species of *Cypripedium*—*C. spectabile, C. parviflorum, C. pubescens,* and *C. candidum*. She claimed that, after the division of the megaspore mother cell, the upper dyad cell promptly degenerates, while the nucleus of the lower cell divides twice to form four nuclei, two lying at each pole of the sac. No further divisions were said to occur; the two nuclei at the micropylar pole organize into two synergids, and the chalazal pair migrates upward to form the egg and a polar nucleus. Subsequently, at the time of fertilization, the nucleus of one of the synergids loosens itself and migrates downward, fusing with the single polar nucleus and the second male gamete to form the primary endosperm nucleus. This mode of development, which began to be known as the *Cypripedium*-type, was generally believed to be correct until RUTGERS (9) put forward some strong objections against it and suggested a reinvestigation.

From a study of PACE's illustrations, RUTGERS offered the following alternative interpretation: After the 4-nucleate stage is reached, the upper of the two daughter nuclei at the micropylar pole divides again, resulting in the formation of three nuclei at this end. Of these, the two sister nuclei of the last division give rise to the two synergids and the lower nucleus constitutes the egg. The two chalazal nuclei remain undivided and move up to function as the polars.

While the objections put forward by RUTGERS against the interpretation of

PACE are no doubt justified, his own interpretations give rise to fresh difficulties. There is not a single authentic instance to indicate that both polar nuclei may be derived from the same end of the embryo sac, nor is there any evidence that only one of the two micropylar nuclei (at the 4-nucleate stage) may divide again while the other remains dormant. Three cases of this kind are those of *Garcinia kydia* and *G. treubii* studied by TREUB (13) and of *Moringa oleifera* investigated by RUTGERS himself. Here the embryo sacs are monosporic and the development proceeds normally up to the 4-nucleate stage, but only one of the micropylar nuclei is said to divide further, while the remaining micropylar nucleus and the two chalazal nuclei remain undivided. The resulting embryo sac is thus 5-nucleate. PURI (7, 8) demonstrated the untenability of this hypothesis and showed that the embryo sac is really 8-nucleate and develops normally, the deceptive appearance being caused by an early degeneration of the antipodal cells. The only difference between RUTGERS' interpretation of PACE's figures and his own account of *Moringa*—as well as that of TREUB on *Garcinia*—is that in *Cypripedium* we are dealing with a bisporic embryo sac and in the other two genera with a monosporic one. RUTGERS, however, admits that his interpretation of PACE's figures is "a possible explanation" and not a "decision," which can only be arrived at by a reinvestigation.

PROSINA (6) investigated *C. guttatum* and found that the embryo sac develops according to the *Allium*-type, and also that the primary chalazal nucleus may

either remain undivided or divide only once, so that the chalazal part has only one or two nuclei instead of the normal four. Fertilization is reported as normal. FRANCINI's (2) studies on some species of *Paphiopedilum*, another genus of the Cypripedilinae, show a similar type of development.

In view of these differences and uncertainties, SCHNARF (10–12) and MAHESHWARI (4) suggested a reinvestigation of the species studied by PACE. This seems to have been undertaken by CARLSON (1), but the only paper so far published by her[1] does not contribute much toward the solution of the problem—beyond her statement that in many of her preparations she saw more than four nuclei in the embryo sac.

Material

When the late Professor M. A. SAMPATHKUMARAN returned to India from Chicago about the year 1918, he brought with him some inbedded material of *C. spectabile*, which he very kindly turned over to the writer shortly before his death. From the label on the wrapper I gather that it was given to him by the late Professor C. J. CHAMBERLAIN when he was a student of the latter at the University of Chicago. As *C. spectabile* happens to be one of the species on which PACE based her original observations and interpretations, I gladly undertook to reinvestigate it and record here my appreciation of the opportunity afforded by this material.

Observations

My observations on the structure of the ovule and the development of the embryo sac up to the 2-nucleate stage

[1] Since this paper by SWAMY was accepted for publication, an article on *Cypripedium* has appeared by CARLSON (BOT. GAZ. 107:107–114. 1945).

are in close agreement with those of PACE. The megaspore mother cell on division gives rise to two dyad cells, of which the upper regularly degenerates while the nucleus of the lower divides to form two nuclei unseparated by a wall or membrane (fig. 1). These move apart to opposite poles, and, at the time they embark upon the next division, a large vacuole makes its appearance in the center of the sac (fig. 2). Frequently the primary micropylar nucleus divides first, while the chalazal one remains dormant for some time (fig. 3). Usually the latter divides only once (fig. 4); one of the two daughter nuclei then functions as the lower polar nucleus and the other is to be regarded as the single antipodal. In rare cases one or both nuclei were found to go through the next division, forming three or four nuclei at the chalazal end. In the latter case three antipodals can be seen in the lower end of the embryo sac (fig. 6).

The primary micropylar nucleus regularly undergoes two divisions to form four daughter nuclei. The two spindles are at right angles to each other, and the available evidence indicated that the two synergids on the one hand and the egg and upper polar nucleus on the other are formed from sister nuclei.

The two polar nuclei usually fuse before fertilization (fig. 5). In a few cases both the chalazal nuclei, one being the lower polar nucleus and the other the single antipodal, were found lying in close juxtaposition with the upper polar nucleus.

The pollen tube enters the micropyle and touches the embryo sac close to the point of insertion of the egg apparatus. Here it pierces the embryo-sac membrane, demolishing one of the synergids lying in its way and discharging the two
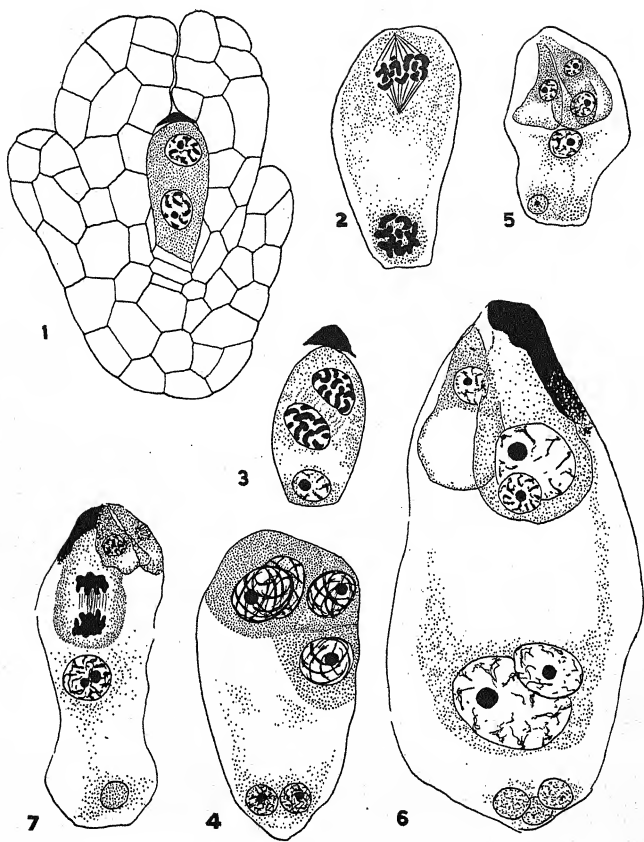
FIGS. 1–7.—Fig. 1, 2-nucleate embryo sac. Fig. 2, division of two nuclei of embryo sac. Fig. 3, division of primary micropylar nucleus completed; primary chalazal nucleus still in resting stage. Fig. 4, 6-nucleate embryo sac. Fig. 5, mature embryo sac after polar fusion. Fig. 6, double fertilization. Fig. 7, first division of zygote; note persistence of both synergids in this case.

sperm nuclei into the sac. The other synergid persists for a while, and in rare cases both synergids seemed to remain intact for some time (fig. 7). Particular attention was paid to ascertain whether one of the synergid nuclei migrated downward to take part in triple fusion, as supposed by PACE, but no such evidence could be found. Figure 6 shows that double fertilization takes place normally.

Summary

1. The embryo sac of *Cypripedium spectabile* develops according to the *Allium*-type; and up to the 2-nucleate stage, my observations are in agreement with those of PACE.

2. The primary micropylar nucleus now divides twice to form the four micropylar nuclei, which typically organize into two synergids—the egg cell and the upper polar nucleus. The primary chalazal nucleus usually divides only once, but sometimes one or both of the resultant nuclei divide once again, resulting in 6-, 7-, or 8-nucleate embryo sacs of the kind seen in many other members of the Orchidaceae and certain of the Alismaceae (3). In all cases one nucleus from the chalazal group functions as the lower polar nucleus. The respective interpretations of PACE, RUTGERS, and the writer are shown in figure 8.

3. No indication could be found in favor of PACE's supposition that during fertilization one synergid nucleus moves downward and enters into triple fusion
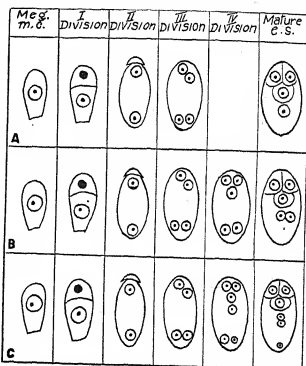
FIG. 8.—Diagrammatic representation of mode of development of embryo sac of *Cypripedium*, according to the interpretations of *A*, PACE (1907); *B*, RUTGERS (1923); and *C*, present writer.

with the polar nucleus and the second male gamete.

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CURRENT LITERATURE

Physical Chemistry of Cells and Tissues. By RUDOLPH HÖBER; with the collaboration of DAVID I. HITCHCOCK, J. B. BATEMAN, DAVID R. GODDARD, and WALLACE O. FENN. Philadelphia: Blakiston Co., 1945. Pp. 676. Illustrated. $9.00.

The reappearance in completely new form of HÖBER's well-known work, *Physikalische Chemie der Zelle und der Gerwebe*, the last edition of which was published in 1926, is important to all who need to interpret the behavior of living cells or organisms in terms of physico-chemical principles.

This new book is written in eight sections, each the work of one of the five authors: Professor HÖBER himself, DAVID I. HITCHCOCK, J. B. BATEMAN, D. R. GODDARD, and W. O. FENN, who have combined to cover this extensive field of knowledge.

The first two sections treat concisely the physico-chemical principles which bear upon cellular phenomena. The emphasis here is upon the great strides made in the last decades in the elucidation of the submicroscopic structure of matter and the nature of the forces which bind particles together to form the units, large and small, the fibers, fibrils, surface films, and membranes which constitute the building units from which cellular architecture is derived. Diffusion, reaction velocities, the elements of thermodynamics, the properties of solutions, and the principles which underlie the phenomena of bioelectric potentials are all concisely reviewed.

Sections 3, 4, and 5, written by HÖBER himself, are concerned with the permeability properties of cells, and here the illustrative material is drawn in large part from the study of plant cells and includes a synopsis of the definitive works of COLLANDER and his school. The evidence which has made it necessary to visualize the surface of the protoplast as partaking simultaneously of permeability properties due to lipoid solubility and of properties due to passive diffusion through minute water-filled pores is reviewed.

Cell respiration is a field in which the student and the investigator must often ignore the somewhat arbitrary division between animal and plant material. The section devoted to a condensed review of this aspect of the subject is particularly interesting because it leads up to the final sections, which deal with mechanisms by which cells and tissues utilize their energy of metabolism to perform work.

The section dealing with contractile tissues in general, and muscle in particular, is naturally concerned almost wholly with animal systems; but the newer trends in this extensive subject are of interest to botanists, inasmuch as they indicate the possibility that the energy used by muscle in the performance of external work may be localized in specific reactions, and there is evidence that the energy liberated by oxidation may be stored up in phosphorylated compounds and transferred from compound to compound with the "high-energy phosphate" grouping.

HÖBER's concluding section, which deals with problems of "active transport" in plant and animal systems, is the climax of the book. After surveying the field, HÖBER authoritatively recognizes that it is a widespread function of living cells to "secrete" water or salts in a manner which demands that they must use energy to perform osmotic work. "Secretion" by the plant protoplast internally to the vacuoles is a classic case in point. This important problem in plants is treated as part of an even more general one, of which other examples are drawn from a wide range of biological systems. In all of them, however, the basic problem remains the same —how is the energy of metabolism used to perform osmotic work?

This book constitutes the most authoritative recent account of cells and tissues regarded as physico-chemical systems. Doubtless it will be freely consulted by cell physiologists for a long time to come. The subject matter, compactly contained in 635 pages, is liberally indexed and supplied with references to recent literature, either as footnotes or in lists of general references at the end of chapters, and the book itself is attractively produced.—F. C. STEWARD.

Fungicides and Their Action. By JAMES G. HORSFALL. Waltham, Mass.: Chronica Botanica Co.; New York: Steckert & Co., 1945. Pp. 239. Figs. 24. $5.00.

This second volume of the *Annales Cryptogamici et Phytopathologici* will be of special interest to those engaged in waging war against those fungi which injure and kill crop plants and to those interested in understanding the mechanisms and dynamics whereby fungicides bring about their injurious and killing effects.

The volume carries a foreword by DAVID FAIRCHILD, with an interesting reminiscence about the days when the mycological and fungicidal pattern of American phytopathology was set and when ALFRED FISCHER "denied that there are any such things as plant diseases caused by bacteria."

The author conceives of fungus control as warfare which is confronted with two basic problems— "(a) to procure the proper materials for killing the enemy, and (b) to deliver them to him in sufficient quantity when and where he is vulnerable." He focuses his task as a discussion of "these two problems in the light of chemistry and physiology of toxic action and of the mechanics of application." He succeeds well.

Chapter I is devoted to a historical introduction. Recorded use of fungicides goes back to the ancient Greeks, showing how mankind often hits upon the right track without understanding. The ancient Greeks knew nothing about the enemy they were combating with fungicides. Chapter II takes up definitions and basic concepts. These non-technological chapters are the least satisfactory part of the volume.

The remaining fourteen chapters present a satisfactory survey of the diffuse knowledge of one of the frontier fields of phytopathology. As the reviewer read these chapters he was again impressed by the wonderful opportunity which investigation of the injurious and killing effects of fungicides on fungi presents for study of the physics and chemistry of injury and death in plants. Viewed in this light, this volume should interest others than those professionally employed or interested in fungicidal control of fungus diseases in crop plants.

The volume closes with a comprehensive bibliography, a general index, and an authors' index. Its physical form is up to the excellent standard of the Chronica Botanica publications.—G. K. K. LINK.

An Introduction to the Taxonomy and Nomenclature of Fungi. By G. R. BISBY. Kew, Surrey: Imperial Mycological Institute, 1945. Pp. vii+117. $1.25.

As its title suggests, this little book is intended primarily for students who are beginning the study of systematic mycology. Following a brief introduction and discussion of principles, the remainder of the text is comprised under two parts—taxonomy and nomenclature. Under the first part a number of suggestions, many of them based directly on the personal experiences of the author, are presented on such topics as equipment, collecting, examining and recording, culturing, naming and describing, preserving, and publishing and illustrating.

In part 2 are given brief discussions of categories of fungi, synonomy, types and the type method, diagnoses, and rules. This part should prove helpful, not only to beginners but also to other botanists interested in nomenclature, since the International Rules of Botanical Nomenclature, not readily available in many libraries, are here presented in full.—J. M. BEAL.

Bibliography of References to the Literature on the Minor Elements, and Their Relation to Plant and Animal Nutrition. 6th Supplement to 3d ed. New York: Chilean Nitrate Educational Bureau, Inc., 1945. Pp. 103.

In this supplement are found more than 700 abstracts and references. These are concerned with 52 chemical elements and 95 plants and plant groups. The four indexes of the fifth edition—author, element, animal nutrition, and botanical—are continued. The yearly issuance of the supplement is helpful to those wishing to keep abreast of tleh iterature of mineral nutrition.—S. V. EATON.

EFFECTS OF NAPHTHALENEACETIC-ACID SPRAYS ON THE DEVELOPMENT AND DROUGHT RESIST- ANCE OF PINE SEEDLINGS

T. E. MAKI,[1] HUBERT MARSHALL,[2] AND CARL E. OSTROM[3]

Introduction

The original discovery that plant-growth regulators produce an inhibiting effect on bud development opened a field of research with much possible practical application. In forest-nursery practice alone, at least three potential uses are suggested on the assumption that the inhibitory effects can be produced without injury to seedlings. First, seedling growth characteristics might be sufficiently altered to result in plants possessing small tops but normally developed root systems. Such plants should be more drought resistant because of the reduced likelihood of excessive transpiration during the period of root establishment. Second, the dormant period of seedlings might be prolonged several weeks in the spring, thereby extending the planting season in regions where environmental conditions are favorable but where the extent of new foliage development determines the end of the planting season. And third, overall top development of seedlings might be sufficiently retarded to permit a year's postponement of lifting, thus making it feasible to save valuable stock when manpower shortages, unfavorable weather, or other circumstances preclude planting during the scheduled year.

These potentialities for regulating the development of forest nursery stock stem from the original discovery that

externally applied growth regulators inhibit the bud development of herbaceous plants. Later investigations have dealt with woody plants, including a few tree species, but no work has been reported on seedlings commonly used in forest planting. To investigate the feasibility of using plant-growth regulators in nursery practice, a number of exploratory studies[4] were undertaken at the Beltsville Forest Laboratory, a branch of the Northeastern Forest Experiment Station located near Laurel, Maryland. Results of the first tests, begun in 1942 and early 1943, have been summarized by OSTROM (11). The present experiment was undertaken in the fall of 1943 to determine the effect of concentration of one growth regulator and season of application on shoot development of pine seedlings. Although this experiment has not provided recommendations for nursery practice, the results may be of interest to those exploring other facets of the problem.

Review of literature

THIMANN and SKOOG (14) reported that inhibition of lateral buds by actively growing terminal shoots is due to the production and downward transport of auxin from the terminals. Others have reported that the resumption of growth by dormant buds of perennial plants is as-

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sociated with a rapid increase in the auxin content of buds and nearby cambium layers (1, 18). Likewise, several investigators have found that relatively low concentrations of synthetic growth regulators may stimulate resumption of vegetative growth in dormant buds (2, 4, 5, 15).

On the other hand, there is abundant evidence that stronger concentrations may prolong both floral and vegetative dormancy of woody plants. Apparently the first to attempt this was WINKLEPLECK (16, 17), who sprayed peach trees with 125 mg. of naphthaleneacetic acid per liter of water when the fruit buds were beginning to break. Sixteen days later unsprayed trees were in full bloom, but sprayed trees did not reach that stage for an additional 11 days. In similar, though more extensive, experiments, MITCHELL and CULLINAN (9) failed to corroborate these results. They sprayed detached branches of pear and peach with naphthaleneacetic acid generally ranging from 100 to 300 mg. of acid per liter of lanolin emulsion or fish-oil spray. Results showed that treated floral buds were stimulated to open in greater numbers and several days earlier than controls. The same treatments delayed the opening of vegetative buds, however, and the suggestion is made that this response might be exploited to advantage in transplanting trees and shrubs.

HITCHCOCK and ZIMMERMAN (3) found that growth-regulator sprays applied in the fall delayed the breaking of dormancy in the spring more effectively than similar sprays applied when the cold-period requirement was satisfied. Subsequently, they found that the opening of both flower and vegetative buds of several varieties each of apple, cherry, plum, pear, and peach was delayed by the application of aqueous sprays con-

taining 200, 400, or 800 mg. of potassium α-naphthaleneacetate per liter of solution applied in July, August, or September of the previous growing season. The intensity of response was greater on the earlier spraying dates and with the higher concentrations. Optimum treatments delayed the opening of flower buds 14 days and vegetative buds 19 days. These results suggest that growth regulators apparently prolong dormancy by affecting in some manner the rate or nature of bud development in the season prior to normal florescence or foliation.

Several other techniques have been employed to prolong the dormancy of buds. SELL et al. (12) painted tung buds two to four times at 2-week intervals with either 0.3% indoleacetic acid or 0.3% naphthalene acetamide carried in a lanolin emulsion and found that dormancy was prolonged for a full 2 weeks. Two other methods—injecting aqueous solutions of growth regulators directly into the buds, or spraying them on the buds in an oil carrier—failed to produce the desired results. Later it was found (13) that lanolin emulsions, in the absence of growth regulators, significantly prolonged dormancy and that only naphthaleneacetic acid and related compounds prolonged it beyond that caused by lanolin alone. Single and repeated applications of naphthaleneacetic acid produced inhibition roughly proportional to the concentration of the acid and the earliness of application. In all tests, however, the prolonging of dormancy was associated with mortality of treated buds, and it is concluded that none of the treatments was satisfactory for orchard use.

Lastly, in an extensive piece of work, MARTH (4, 5) tested the effectiveness of nineteen chemical compounds for prolonging the vegetative dormancy of rose

bushes and other woody plants held in common storage. Growth regulators were applied as vapors or as sprays carried in a 0.25% wax emulsion. Spray applications of seventeen growth regulators in concentrations of 0.05, 0.01, and 0.005% showed that most of the substances were effective in promoting complete dormancy during 40–60 days of unrefrigerated storage. Controls during the same period put on so much new shoot growth that subsequent survival and development in the field was not equal to that of treated plants. β-indolepropionic acid, tetrolin-6-acetamide, and phenylacetic acid, three of the substances most ineffective for prolonging dormancy at even 0.05%, definitely stimulated bud development at 0.005%, whereas the more potent compounds like naphthaleneacetic acid caused no stimulation. It may be that, with the proper concentration, any growth regulator might stimulate bud development at sufficiently low strengths, but at higher concentrations it might effectively prolong dormancy.

The tests with vapors were made on a number of varieties of roses, on several varieties each of *Amygdalus, Prunus, Pyrus,* and *Malus,* and also on *Acer palmatum, Diospyros virginiana,* and *Philadelphus grandiflorus.* These tests showed that exposure to the vapors of naphthalenemethylacetate at concentrations of 0.3–0.5 gm. per 1000 cubic feet of air for 16 hours at 70° F. was sufficient to hold most plants dormant for storage periods ranging from 1 to 2 months. Lowering the concentration to 0.1 gm. per 1000 cubic feet, however, again produced bud stimulation which frequently resulted in shoot growth far surpassing that of control plants.

No reports dealing with species and age classes used in forest nurseries were found in the literature. Accordingly, exploratory tests (11) on nursery seedbeds of loblolly, red, and table-mountain pines were undertaken at the Beltsville Forest Laboratory in 1942 and 1943. Sprays carrying 200 mg. of naphthaleneacetic acid per liter of lanolin emulsion, or 200–600 mg. of naphthalene acetamide per liter, or the same total concentrations of a mixture (equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid) were applied when the buds were beginning to elongate. These sprays failed to prevent the initial surge of leader growth but frequently caused a reduction of more than 50% in the number of new needles. Sprays applied when needles were elongating usually arrested needle growth at about the stage existing when the spray was applied. Treatments which produced definite inhibition of new leader growth often killed some of the stock, and a repeat application of spray was generally lethal. At least part of this mortality was attributed to the excessive weight of the carrier (50 gm. of lanolin per liter of emulsion), as contrasted with normal carrier weights of 5–10 gm. of lanolin. Leaders of loblolly and table-mountain pines inhibited by high concentrations usually were curved, but red pine leaders remained straight. Loblolly pines inhibited by spring treatment frequently resumed leader growth during the summer.

These nursery seedbed treatments indicated that spring and early summer applications were not particularly effective for controlling leader elongation, even though causing a decrease in the top-root ratio and retarding to some extent increases in dry weight. However, it seemed desirable to test fall and early spring applications in order to determine whether these might be more effective

for controlling the initial surge of leader growth. Accordingly, an experiment was begun in September, 1943, to test the inhibiting effects of naphthaleneacetic acid in varying concentrations on four species of pine widely used for forest planting purposes.

Experimental procedure

INITIAL NURSERY TREATMENTS

The treatments applied to seedlings in the nursery beds consisted of spraying with α-naphthaleneacetic acid in five concentrations—200, 400, 600, 800, and 1000 mg. per liter of 1% Dowax emulsion, and top-pruning to a height which removed most of the terminal buds and approximately half the foliage, no spray being applied to the pruned seedlings. Pruning was included simply to compare a known method of reducing top growth with the possible retarding effects of the growth regulator. Species and age classes were as follows: 1-year jack pine (*Pinus banksiana* Lamb.), 1-year loblolly pine (*P. taeda* L.), 2-year white pine (*P. strobus* L.), and 3-year red pine (*P. resinosa* Ait.).[5] The complete series of treatments was repeated on September 23 and October 20, 1943, and March 11, 1944.

The arrangement of treatments in the nursery beds was systematic rather than random, 3-foot segments of the bed being assigned to each of the five spray concentrations, to the top-pruning treatment, and to untreated controls. Within each 3-foot segment, 1-foot strips extending across the bed were assigned to each of the three spraying and pruning dates. Since the beds were 4 feet wide, each unique treatment combination was applied to 4 square feet of bed space. This

area was sufficient to provide about 150 seedlings that were large and thrifty enough for subsequent planting in the field.

The 1-foot strips were separated by lath laid across the bed and in contact with the soil. Before spraying, a length of cardboard was inserted vertically along the lath-line and bent outward to protect adjacent seedlings from contamination. The spraying consisted simply of applying 200 cc. of the emulsion carrying the proper concentration to each 4 square feet of bed, by means of a small hand-sprayer of 1-quart capacity. The operation was somewhat hampered by the fact that Dowax in the concentration used tends to be precipitated by the alcoholic solution of naphthaleneacetic acid, the small particles of wax occasionally clogging the apparatus. With mechanical agitation, however, the consistency of the emulsions was generally improved and the operation resulted in a fairly uniform application.

LATER TREATMENT OF STOCK

Shoot growth of jack pine began in the spring of 1944 during the first week in April, and by May 1 the initial surge was practically complete. By that time it was apparent that the only treatments causing appreciable retardation of shoot growth were the September sprays at 800 mg. and 1000 mg. per liter and top-pruning. Accordingly, on May 1 groups of 108 jack pine seedlings were lifted from the control segment, the September top-pruned strip, and the strip sprayed with 800 mg./l. of acid in Dowax emulsion, the October and March segments being left intact in the beds. The lifted seedlings were used to determine the growth characteristics and relative drought resistance of the three September-treatment groups. The seedlings of

each group, however, were subdivided into three reasonably discrete size classes of thirty-six seedlings each, the basis for grading being the degree of inhibition of new growth. This procedure provided a means of comparing the relative drought resistance of seedlings treated in the same manner but differing in degree of inhibition.

Immediately after grading, three measurements were taken on each seedling: (*a*) diameter at the cotyledon scar, (*b*) old shoot length measured from the cotyledon scar to the base of the current season's growth, and (*c*) new shoot growth to May 1.

On May 9, the jack pine seedlings were planted in sand in a greenhouse to determine whether the spraying or top-pruning had affected drought resistance. Three blocks were planted, each containing all nine treatment combinations in randomized order. The sand was thoroughly watered immediately after planting, but no additional water was added for the duration of the test. Periodic observations on the condition of the seedlings were made to record relative resistance to drought.

In contrast to jack pine, loblolly pine made practically no growth in April; however, by the third week in May new leader growth averaged about 5 inches. As in jack pine, it was strikingly demonstrated that only the September spray applications and the top-pruning treatment had been effective in retarding top development (figs. 1, 2). Accordingly, on May 18, 1944, about eighty-five plantable seedlings, representing 2 square feet of bed space, were lifted from each 1-foot strip of bed treated the previous September, with the exception of the control segment—from which was lifted twice this number. Since top development in loblolly begins by elongation of the bud

to form a "candle," which in the early stages is devoid of leaves, it was possible to utilize this characteristic in assessing the degree of inhibition caused by the spray treatments. The grading criteria for loblolly pine were therefore set up as follows:

Uninhibited: Buds definitely elongated into candles which were already putting forth new needle growth.
Partly inhibited: Average candle elongation about $\frac{3}{4}$ inch but needle growth not yet started.
Inhibited: Buds showing signs of swelling in most instances but no elongation.

These criteria naturally caused the formation of groups containing unequal numbers of trees. For example, seedlings treated with the two highest concentrations of naphthaleneacetic acid fell largely into the inhibited and the partly inhibited classes, while the entire lot of controls fell into the uninhibited class. These three classes again provided a basis for comparing the relative drought resistance of seedlings treated in the same manner but differing in degree of inhibition.

After grading, the same measurements as for jack pine were taken on each seedling, with the exception that old shoot lengths were not measured on the top-pruned lot, since—theoretically at least—this length was uniform. On May 26 the seedlings were planted in a sandy area of the Beltsville forest nursery in an attempt to determine whether any of the five spray applications or the top-pruning treatment had caused differences in drought resistance. A 4-block planting arrangement was used, each of the treatments being randomized within the blocks.

Neither red nor white pine showed any marked response to spray treatment, except for slight inhibition of shoot

growth in the latter resulting from the two highest concentrations of the acid applied in September. Top-pruning, of course, resulted in a marked reduction in seedling heights of both species; but, since the response to growth regulators was so slight, no detailed records or measurements were made on either species.

refers to the total first year's (1943) shoot length; new growth refers to leader elongation in the second growing season (up to May 1, 1944). Equations for the regression of length of new leader over length of old shoot within each treatment group are plotted in figure 3; they show the effect of the treatments on seedlings which differed in initial size.
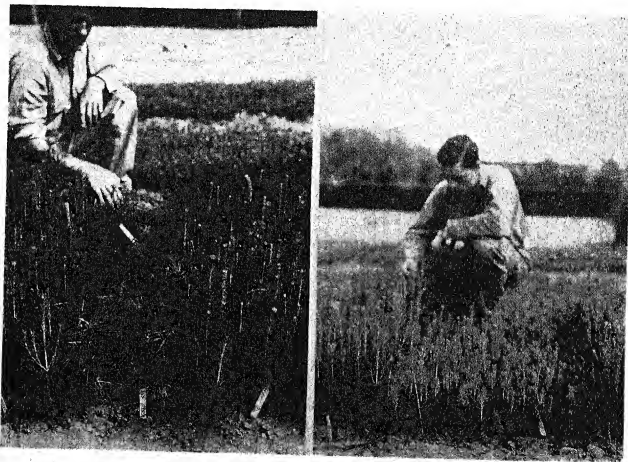
FIGS. 1, 2.—Fig. 1 (left), inhibition of shoot growth of 1-year loblolly pine resulting from naphthalene-acetic-acid spray at 800 mg./l. applied to 1-foot-wide strip of bed, September 23, 1943. When photographed on May 18, 1944, an occasional seedling showed some yellowing of needles, but no mortality was evident. Labels in foreground are $3\frac{1}{2}$ inches long. Fig. 2 (right), retardation of leader growth in loblolly pine resulting from top-pruning bed strips (1 foot wide) at about 4 inches above cotyledon scar on September 23 and October 20, 1943. No discernible differences between pruning dates were found on May 18, 1944, when photograph was taken. Average length of new leader in pruned stock, $1\frac{3}{4}$ inches; in unpruned stock, about 5 inches.

Results

JACK PINE

GROWTH CHARACTERISTICS.—A comparison of old and new shoot growth by treatments and by degree of inhibition based on amount of second-year leader growth is shown in table 1. Old growth

New growth in control seedlings varies directly with old shoot length. This positive relationship is characteristic of many species of coniferous seedlings in the second growing season. Both the top-pruning and the top-spraying treatments materially altered the normal pattern of shoot development.

Top-pruning caused a complete reversal when compared with the relationship of new to old shoot growth in the unpruned trees; that is, the larger seedlings after pruning actually produced less new leader growth than the small ones. Although not proved experimentally, this relationship may have arisen mainly from the shorter seedlings that escaped decapitation during the pruning.

lings than for small ones, probably because the smaller seedlings were partially protected from the spray by the larger ones in the crowded nursery beds.

The means of all sizes in table 1 (see also fig. 4) give a picture of the average response of run-of-the-bed seedlings which might ordinarily be used in planting. It is apparent that, despite the slightly larger original size of sprayed

TABLE 1

MEAN FIRST YEAR'S SHOOT LENGTH (OLD) AND SECOND YEAR'S LEADER
GROWTH (NEW) OF JACK PINE SEEDLINGS ON MAY 1, 1944
BASIS: 36 SEEDLINGS PER MEAN

SEEDLING SIZE CLASS*	AGE OF SHOOT GROWTH	AMOUNT OF SHOOT GROWTH (INCHES) BY TREATMENTS					
		Control		Top-pruned 9/23/43		Top-sprayed 9/23/43 (800 mg./l. nac.)	
		Mean	SE	Mean	SE	Mean	SE
Large...........	Old (1943)	2.46	±0.0758	1.52	±0.0799	2.42	±0.113
	New (1944)	4.85	.0956	2.45	.0534	2.58	.0877
Medium.........	Old (1943)	2.00	.0952	1.90	.0750	2.26	.113
	New (1944)	3.79	.0836	1.85	.0484	1.46	.0531
Small...........	Old (1943)	1.57	.0832	1.77	.0762	2.36	.132
	New (1944)	2.83	.0912	0.61	.0602	0.78	.0385
Means of all sizes	Old (1943)	2.01	.0602	1.73	.0466	2.35	.0686
	New (1944)	3.82	±0.0951	1.64	±0.0801	1.60	±0.0806

* Based on length of new (1944) growth.

ing. These unpruned seedlings had the advantage of producing single dominant leaders from the terminal buds, whereas the larger pruned seedlings were forced to resume growth from lateral buds along the stem. Usually several lateral buds developed, thus dividing the "growth energy" of the plant and temporarily reducing the rate of elongation as compared with single terminal shoots.

The spray treatment likewise altered the pattern of top development. Table 1 and figure 3 indicate that inhibition of shoot growth was greater for large seed-

seedlings, their leader growth up to May 1 was less than half (42%) that of controls. New leader growth of top-pruned seedlings was reduced practically the same amount, but this reduction was at the expense of foliar surface.

In contrast to the marked effect of treatments on shoot lengths, measurements of stem diameter revealed no differences between treatments which could not be ascribed largely to initial differences in size of seedlings.

DROUGHT RESISTANCE.—Jack pine seedlings were planted in the greenhouse

on May 9, 1944, after the various measurements had been made. Temperatures in the greenhouse were severe, ranging between 90° and 110° F. on mid-afternoons of bright days; and, since no water was added to the sand subsequent to the initial moistening, the seedlings soon showed evidence of drought stress. By June 10 approximately half of them were either dead or showing unmistakable signs of injury. Accordingly, a tally was taken to determine differences in vigor (table 2).

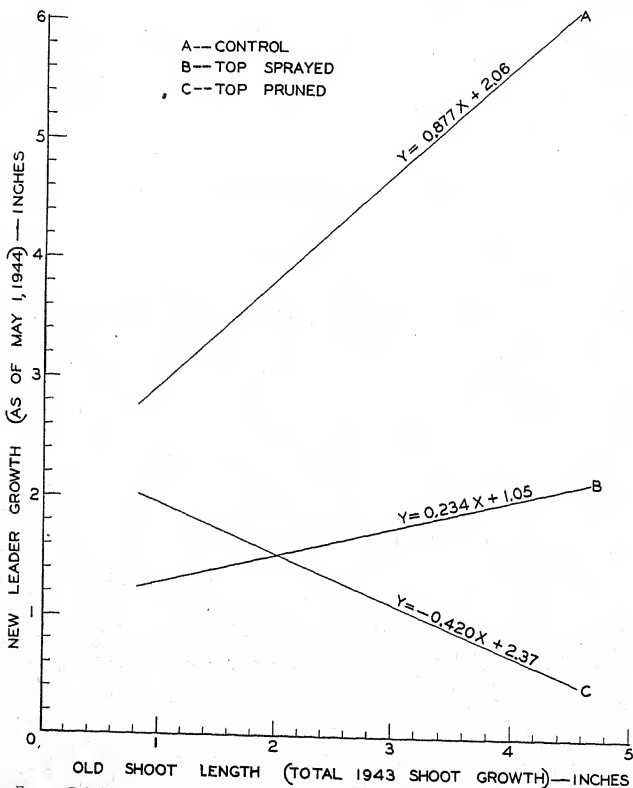Top-pruning and spraying with naph-

FIG. 3.—Relation of new leader growth to old shoot length for jack pine treated as shown. Regression coefficient of controls significant at 0.01 level; of treated groups at 0.05 level.

thaleneacetic acid in September at a concentration of 800 mg./l. reduced the drought resistance of jack pine. Since the three grading groups were based upon the amount of new leader growth produced during April, 1944 (not total height), it becomes clear that hardiness to drought was largely associated with the amount of existing new shoot growth. This was particularly true of sprayed seedlings, where only four of the heavily inhibited group could be classed as thrifty. On the other hand, sprayed and top-pruned seedlings exhibiting a "medium" or "large" amount of new growth survived practically as well as controls; these seedlings, however, had less new growth than even "small" controls (table 1). It is apparent, then, that the substantial reduction in new growth (more than 50 %) shown by the medium and large groups resulted neither in injury nor in immediate benefits during the period in which the plants were subjected to a limited moisture supply.

LOBLOLLY PINE

GROWTH CHARACTERISTICS.—The response of loblolly was similar to that of jack pine, any differences being largely

TABLE 2

NUMBER OF THRIFTY JACK PINE SEEDLINGS ON JUNE 10, 1944, 1 MONTH AFTER PLANTING IN SAND* IN GREENHOUSE. BASIS: 36 ORIGINAL SEEDLINGS PER TREATMENT-SIZE COMBINATION

TREATMENT	NO. THRIFTY SEEDLINGS BY SIZE CLASSES†			
	Small	Medium	Large	All sizes
Control............	16	25	18	59
Top-pruned (9/23/43)	11	20	21	52
Spray (nac. 800 mg./l. on 9/23/43).......	4	20	19	43
Total number of thrifty seedlings (all treatments)..	31	65	58	154

* Sand left unwatered for duration of test (after initial watering immediately after planting).

† Based on length of new leader growth as of May 1, 1944.

FIGS. 4, 5.—Fig. 4 (left), effect of growth-regulator spray and top-pruning treatments applied to seedbed of 1-year-old jack pine on September 23, 1943. Seedlings lifted and photographed May 1, 1944. Upper and lower series are merely replicates showing variation in form and size of control and treated stock. Fig. 5 (right), effect of naphthaleneacetic-acid sprays and top-pruning applied September 23, 1943, to 1-year-old loblolly pine seedlings in nursery bed. Practically complete inhibition achieved with the two highest concentrations. Development of single new leader in uninhibited seedlings of spray series was a marked characteristic contrasting with multi-shoot development of larger controls.

associated with differences in growth habits. At the end of the first season, jack pine develops simply an appressed cluster of small juvenile needles, instead of the distinct terminal bud developed by loblolly. The latter species resumes growth in the spring by elongation of the bud into a "candle," which in the early stages is devoid of needles. This charac-

pattern, however, for table 3 shows no relationship between new growth and the length of the older part (1943) of the treated seedlings.

The general means in table 3 afford a comparison of the total effect of treatments on "bed-run" plantable seedlings. All treatments, except the spray with 200 mg./l., substantially inhibited new

TABLE 3

Effect of top-pruning or spraying with naphthaleneacetic acid in September, 1943, on old shoot length and new growth (in inches) of groups of 1-year loblolly pine seedlings. Measurements taken May 18, 1944

Treatment	Shoot growth	Inhibited			Partly inhibited			Uninhibited			Mean		
		Mean	SE	Basis*	Mean	SE	Basis*	Mean	SE	Basis*	Mean	SE	Basis*
Control.......	Old length	.....	.....	.....	.....	.....	.....	4.42	0.128	108	4.42	0.128	108
	New growth	.....	.....	.....	.....	.....	.....	4.22	.156	108	4.22	.156	108
Top-pruned...	Old length	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	New growth	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.83	.187	87
200 mg./l......	Old length	.....	.....	.....	.....	.....	.....	4.80	.115	93	4.80	.115	93
	New growth	.....	.....	.....	.....	.....	.....	4.54	.129	93	4.54	.129	93
400 mg./l......	Old length	5.38	0.794	5	4.64	0.482	12	5.02	.124	72	4.99	.126	89
	New growth	0	0	5	0.87	.132	12	3.93	.162	72	2.30	.192	89
600 mg./l......	Old length	4.65	.232	17	4.63	.334	22	5.27	.164	45	4.98	.135	84
	New growth	0	0	17	0.80	.0721	22	3.39	.166	45	2.02	.187	84
800 mg./l......	Old length	5.34	.185	59	4.59	.381	16	5.19	.489	9	5.18	.159	84
	New growth	0	0	59	0.54	.0555	16	3.06	.277	9	0.43	.107	84
1000 mg./l.....	Old length	5.28	0.162	49	5.48	.240	16	5.32	.237	21	5.33	.117	86
	New growth	0	0	49	0.56	0.0801	16	2.31	0.181	21	0.67	0.113	86

* No. of seedlings.

teristic of loblolly made it desirable, in grading the stock, to use the three criteria—completely inhibited, partly inhibited, and uninhibited. Differences resulting from the various treatments are summarized in table 3. The growth of loblolly pine in the second year is ordinarily proportional to the size of the seedling at the beginning of the growing season; that is, large seedlings produce a large amount of new growth. The spray treatments altered the expected growth

growth, and the two strongest concentrations caused almost complete inhibition during the period when controls were developing leaders more than 4 inches long (fig. 5).

The column on mean old growth in table 3 shows that these values generally increase with increasing concentration of the growth regulator. There seems to be no explanation for this fact other than gradual variation in size of seedlings which was not adequately controlled by

the method of systematic sampling. This accidental trend in no way invalidates the conclusions, however, since it is known that larger seedlings tend to produce greater new growth. Actually it furnishes additional evidence of the effectiveness of the treatments, since as a result of the spray treatments the larger seedlings actually made less new growth.

To obtain additional information on the nature of the response to growth-regulator sprays, equations were calculated to show the regression of new growth on old shoot length. In order to eliminate marked skewness, it was necessary to exclude the data on completely inhibited seedlings, since obviously the y values for this fraction of the stock were zeros. Control seedlings again displayed the characteristic increase in new leader growth with increase in old shoot length (fig. 6). All sprayed seedlings, on the other hand, showed aberration in varying degrees from this expected growth pattern. The group treated with only 200 mg. of the acid per liter of emulsion showed no over-all inhibition and was not significantly different from

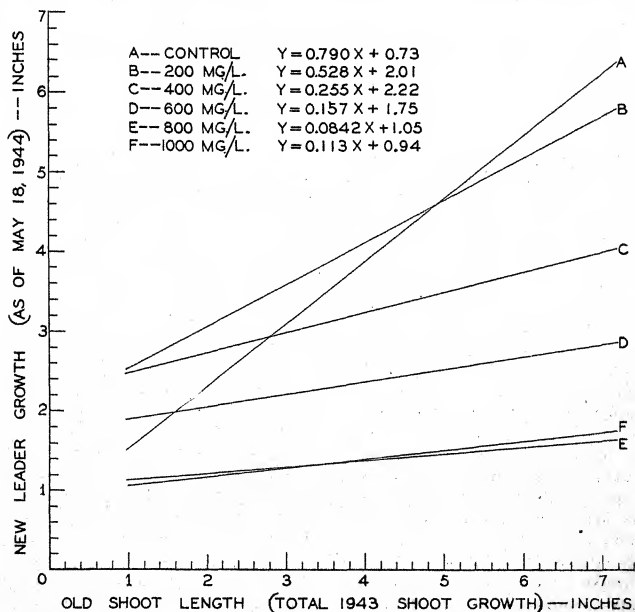| A -- CONTROL | $Y = 0.790 X + 0.73$ |
B -- 200 MG/L.	$Y = 0.528 X + 2.01$
C -- 400 MG/L.	$Y = 0.255 X + 2.22$
D -- 600 MG/L.	$Y = 0.157 X + 1.75$
E -- 800 MG/L.	$Y = 0.0842 X + 1.05$
F -- 1000 MG/L.	$Y = 0.113 X + 0.94$

FIG. 6.—Relation of new leader growth to old shoot length. Data on completely inhibited seedlings omitted to avoid skewness in distribution. Regression coefficients of controls and 200-mg. treatment significant at 0.01 level; other coefficients not significant.

controls. All other groups showed considerable inhibition, the large seedlings being inhibited relatively more than the small ones.

The measurements of stem diameters at the cotyledon scar for loblolly pine are summarized in table 4. Among the sprayed seedlings, those showing little or no inhibition of shoot growth were larger than the controls. The possibility that inhibited seedlings were smaller before treatment seems unlikely, inasmuch as these seedlings had, on the average, as much original shoot length (old

theoretically should average smaller in diameter than controls. Such is not the case. No entirely satisfactory explanation for this incongruity appears possible in the absence of measurements on seedling size prior to treatments. It is possible that growth regulators stimulate diameter increment at dosages below the threshold for complete inhibition, and that this stimulation is inversely related to the degree of inhibition in shoot growth. In contrast to the spray treatments, top-pruning reduced mean diameter growth well below that of controls.

TABLE 4

EFFECT OF TOP-PRUNING OR TOP-SPRAYING WITH NAPHTHALENEACETIC ACID IN
SEPTEMBER, 1943, ON DIAMETERS (IN INCHES) OF 1-YEAR LOBLOLLY PINE
MEASUREMENTS TAKEN MAY 18, 1944

TREAT-MENT	INHIBITED			PARTLY INHIBITED			UNINHIBITED			MEAN		
	Mean†	SE†	Basis*	Mean†	SE†	Basis*	Mean†	SE†	Basis*	Mean†	SE†	Basis*
Control....	.....	....	.....	.....	....	.....	8.95	±0.292	108	8.95	±0.292	108
Top-pruned	.....	....	.....	.....	....	.....	.....	....	.....	8.51	.189	87
200 mg./l..	.....	....	.....	.....	....	.....	9.09	.223	93	9.09	.223	93
400 mg./l..	7.40	±0.400	5	7.71	±0.718	12	10.35	.314	72	9.82	.294	89
600 mg./l..	9.24	.579	17	10.09	.720	22	12.42	.360	45	11.17	.327	84
800 mg./l..	9.69	.258	59	9.38	.523	16	10.44	.580	9	9.71	.216	84
1000 mg./l..	8.23	±0.249	49	9.31	±0.564	16	9.78	±0.440	21	8.81	±0.217	86

* No. of seedlings.   † d_4 inch.

growth) as either of the other two groups (table 3). Apparently the treatments resulting in the heaviest inhibition of shoot growth also resulted in the greatest inhibition of diameter growth.

The differences between treatment means, however, are not so readily interpreted. The close relationship between the inhibition of shoot growth and the inhibition of diameter growth in the sprayed seedlings seems well established. However, the mean diameters for all spray treatments causing inhibition are larger than controls. If inhibition of new leader growth also inhibits diameter growth, the severely inhibited groups

The fresh weight of seedlings was drastically affected by treatment (table 5). Inhibited seedlings, as well as the top-pruned ones, were on the average only about 50% as heavy as the controls.

DROUGHT RESISTANCE.—Drought resistance of loblolly pine was tested by means of a nursery planting installed May 26, 1944. The soil was fairly dry at the time of planting, and hot dry weather prevailed during the succeeding weeks. Mortality and condition of surviving seedlings by treatments, as of June 12, are presented in table 6.

It is evident that loblolly responded to drought conditions in much the same

manner as jack pine. All groups which showed definite inhibition as a result of growth-regulator applications showed lower resistance to drought than did the controls. Spraying with 400 mg./l. resulted in slight injury, and higher concentrations were distinctly injurious. Seedlings sprayed with 200 mg./l., in

sponse can be obtained. The present study, together with the initial tests summarized by OSTROM (11), represents the first reported attempt to inhibit the growth of coniferous stock in seedbeds and is the second to follow the effects of inhibition on behavior of stock after transplanting.

TABLE 5

MEAN FRESH WEIGHT OF UNINHIBITED AND INHIBITED LOBLOLLY PINE SEEDLINGS PRIOR TO PLANTING, MAY 26, 1944

TREATMENT (9/23/43)	MEAN FRESH WEIGHT (GM.)					
	Uninhibited			Inhibited		
	Per seedling	SE	Basis*	Per seedling	SE	Basis*
Control............	7.9	±0.188	8	....	....	......
Top-pruned........	4.1	.189	4	....	....	......
200 mg./l..........	7.1	.112	4	....	....	......
400 mg./l..........	8.5	.135	3	3.8	....	1
600 mg./l..........	10.6	±0.800	2	5.5	±0.300	2
800 mg./l..........	6.4	....	1	5.3	.467	3
1000 mg./l.........	6.4	....	1	4.3	±0.135	3

* No. bundles each containing 20 plantable seedlings.

which new growth appeared somewhat stimulated, survived better than controls. Top-pruned seedlings, although appearing weakest at the time of planting, survived surprisingly well and surpassed controls in number of vigorous seedlings.

Additional tallies were not made, since continued mortality precluded a sufficiently fine evaluation of treatment differences.

Discussion

The marked inhibition of new shoot growth obtained by spraying jack and loblolly pine seedlings with naphthaleneacetic acid confirms the majority of reports in the literature bearing on the subject. Most of the papers dealing with inhibition of the vegetative growth of woody plants have reported that re-

TABLE 6

CONDITION OF LOBLOLLY PINE SEEDLINGS IN NURSERY ON JUNE 12, 1944, 17 DAYS AFTER PLANTING. BASIS: 80 SEEDLINGS PER TREATMENT

TREATMENT (9/23/43)	NO. SEEDLINGS BY CONDITION CLASSES			TOTAL ALL CONDITIONS
	Thrifty*	Doubtful†	Dead	
Control...........	11	27	42	80
Top-pruned.......	17	19	44	80
200 mg./l.........	18	31	31	80
400 mg./l.........	4	27	49	80
600 mg./l.........	4	8	68	80
800 mg./l.........	2	7	71	80
1000 mg./l........	1	1	78	80
All treatments...	57	120	383	560

* Includes all seedlings with fresh new growth and green foliage.

† Includes all seedlings with partly yellow or brown foliage and wilted leaders.

There is close agreement between the results of the present study and those obtained by HITCHCOCK and ZIMMERMAN (3). These investigators found that the intensity of response on a number of varieties of fruit trees varied directly with concentration and earliness of application. The present study is confirming, in that (a) response by jack and loblolly pines was obtained only in the September application, whereas October and March applications were ineffective, and (b) a progressively greater response was usually found with increasing concentrations of the acid.

Three-year-old red pine and 2-year-old white pine seedlings failed to respond to sprays of naphthaleneacetic acid under the conditions of this study. No direct evidence explaining this failure can be offered. However, both species formed terminal buds earlier in the season and both represented larger, huskier, and older plant material than the 1-year-old loblolly and jack pines; hence, it seems likely that inhibition might have been obtained either by higher concentrations or earlier application. Indeed, the earlier tests at Beltsville (11) indicated that red pine at least responded by inhibition of needle growth when treated with top sprays of growth regulators during the period of shoot emergence, although in many instances the responses represented some degree of injury.

The inhibition achieved in the present experiment fulfilled most expectations anticipated from the literature. Early growth was reduced to the extent that treated seedlings at the time of planting had better balance between roots and shoots and considerably less foliar surface to be exposed to desiccating winds. According to the basic assumptions of the experiment, this should have resulted in increased survival, but the opposite was true. Apparently the growth regulator exerted effects together with the inhibition which were more than sufficient to outweigh whatever benefit may have accrued from better-balanced stock. Naphthaleneacetic acid, in concentrations strong enough to cause inhibition of growth, evidently causes serious disturbances in the physiology of the seedling, lowering resistance to drought. Such disturbances have been reported by MITCHELL et al. (6, 7, 8) in kidney beans and by MITCHELL and BROWN (10) in annual morning-glory, which responded to sprayings of growth regulators by marked acceleration of starch hydrolysis and depletion of the supply of readily available carbohydrate reserves.

The only previous investigations of the effects of shoot inhibition on survival of woody transplants are the ones by MARTH (4, 5) and OSTROM (11). MARTH kept untreated and treated rose bushes in common storage until growth on the untreated bushes was well under way. Then, on the assumption that the etiolated and highly succulent new growth would die in the field, he pruned the new growth and planted the stock in the field. Controls, having suffered severe depletion of reserves, failed to survive as well as inhibited plants. OSTROM tried similar treatments on 1-year-old white ash seedlings, except that the etiolated shoots developed during storage were not removed from the plants. After one growing season in the field, treated seedlings showed superior form and vigor, but only slightly better survival, than controls. In the present experiment a reduction of approximately 50% in new growth of jack pine did not materially increase survival after transplanting, and a similar degree of inhibition in loblolly pine was definitely harmful. So far as benefits from inhibition by growth regulators

are concerned, there appears to be a qualitative difference in the responses of evergreen and deciduous species. In the light of these preliminary studies, it does not appear likely that inhibiting treatments with naphthaleneacetic acid can be used to practical advantage for increasing drought resistance of pine seedlings.

Summary

Different nursery beds of 1-year-old jack and loblolly pines, 2-year-old white pine, and 3-year-old red pine were sprayed in September, October, and March with naphthaleneacetic acid in concentrations of 200, 400, 600, 800, or 1000 mg. per liter of 1% Dowax emulsion. Other blocks of seedlings were top-pruned at such a height as to remove most of the terminal buds and about 50% of the foliar surface. Chief results were:

1. Seedlings of white pine and red pine were not perceptibly affected by growth-regulator treatments. An explanation for this failure to respond is offered.

2. Jack pine seedlings sprayed in September with 800 and 1000 mg./l. were inhibited to the extent that on May 1 they had produced only 42% as much new shoot growth as controls. October and March sprayings, and September sprayings of lower than 800 mg./l., produced no discernible effects.

3. Loblolly pine seedlings sprayed in September responded to all concentrations above 200 mg./l., the degree of inhibition increasing with concentration. On May 18, seedlings treated with 800 mg./l. had produced only 10% as much new growth as controls.

4. New growth on top-pruned seedlings of both jack and loblolly pine was restricted almost as much as on the most heavily inhibited seedlings.

5. After being transplanted, both jack and loblolly pine seedlings inhibited with naphthaleneacetic acid showed lower resistance to drought than untreated seedlings. Top-pruned seedlings of jack pine were only slightly inferior to controls, and those of loblolly pine were only slightly better. The only lot showing a slight increase in resistance to drought was loblolly sprayed in September at 200 mg./l.

SOUTHERN FOREST EXPERIMENT STATION
GULFPORT, MISSISSIPPI

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HISTOLOGICAL RESPONSES OF BEAN PLANTS TO PHENYLACETIC ACID

A. GERALDINE WHITING AND MARY AILEEN MURRAY

Introduction

The use of phenylacetic acid as a growth-regulating substance was first mentioned by ZIMMERMAN and WILCOXON (11) in 1935. They found that phenylacetic acid in concentrations of 0.025–3.0% in lanolin would cause negative bending of the tomato stem or epinasty of the leaves, while concentrations of 1.0–3.0% resulted in the induction of adventitious roots. HAAGEN SMIT and WENT (3) also reported that application of phenylacetic acid would result in a slight curvature with the *Avena* test. Since that time, this acid has been used in many experiments. ZIMMERMAN (10) lists it as one of the "physiologically active substances" which cause cell elongation and root initiation. A histological study of the sunflower stem was made by BLUM (1), but he described only an advanced stage of reaction.

The present paper is one of a series (4, 5, 6, 7, 8, 9) on the histological responses of the bean plant to various growth substances. The experiments have been conducted with the same methods and under similar conditions, so that the results are generally comparable.

The phenylacetic acid, a simple aromatic carboxyl acid, was obtained from Eastman Kodak Company and applied in a mixture of 2% acid in anhydrous lanolin. The strain of bean (*Phaseolus vulgaris* var. Red Kidney) and the method of application to the ends of decapitated second internodes are the same as have been previously reported (6). The plants were grown in the spring of 1939 under conditions described earlier (5). Samples were collected at various intervals up to 30 days after treatment, preserved in Navashin's solution, im-

bedded by the butyl alcohol-paraffin method, sectioned at 10–12 μ, and stained in a modified triple stain.

Gross responses

The first response of stem tips to treatment with phenylacetic acid is a change in color to a lighter green for a distance of several millimeters down from the cut surface. This pale green is generally characteristic of responding tissues, except where vigorously proliferating tissues are creamy in color or where suberized or dying tissues darken to yellow or brown.

At the end of 2 days the cut surface is usually concave because of the collapse of central pith tissue. Enlargement of the stem is not distinct until the fourth day, when the diameter at the apex may be half again as large as that of the basal unaffected portion. From this widest level the stem gradually tapers downward for approximately 5 mm. By the sixth day the diameter at the cut surface is about twice that of the lower portion of the stem and varies from 4 to 7 mm. Although stem response continues, further increase in diameter is not marked. The downward extent of the response may reach 8–9 mm. below the cut surface. In many stems the lowest limit of response is evident in a slight shoulder resulting from the abrupt termination of the swelling. The ridges about the major vascular bundles in the flared stem tip are much enlarged, in contrast to the faintly discernible ridges at lower levels.

Tumor development above the original level of the cut surface is first evidenced on the fifth day by a ring of tissue pushed upward between the periphery and the concave center of the stem. Maximum development of the tumor seems to occur between 11 and 15 days

after treatment. Growth may continue for some days more, but in many cases the tumor or parts of it become darker in color and rather shrunken in appearance, indicating cessation of activity and drying out of tissues. By the thirtieth day after treatment many stems and tumors are dead.

The characteristic shape of a tumor is a flat-topped dome supported on a flared and enlarged stem tip. A large tumor at 14 days measured 9 mm. across and 3 mm. in height. Others were generally smaller. In some cases the depression over the central pith is filled by the proliferated tissue. Likewise, peripheral portions of the tumor may grow outward and extend downward around the stem tip. The whole surface of the tumor is markedly irregular, with small round protuberances. These result from irregular proliferation of tissue at the surface of the tumor rather than from organization of root primordia. Rarely, root primordia may be seen near the upper surface of the tumor.

Histological responses

Detailed histological examination shows considerable variation in the response of stem tissues to phenylacetic acid. The proximity to or the distance from the treated surface determines the degree, and in part the character, of the reaction. The maximum proliferation of the stem occurs about 1 mm. below the surface of application. Beyond this the degree of response is in inverse proportion to the distance from the cut surface. The effect is also greater in the areas of the major vascular bundles and less over the smaller ones. Among individual plants, both the degree and the time of reaction vary considerably.

No response was observed in the epidermis, although near the cut surface

there may be some transverse divisions of the cells. The tissue is not ruptured, but by cell enlargement and radial divisions keeps pace with the proliferation of inner structures.

Enlargement of the cells of the cortical parenchyma occurs by the second day and ultimately extends to as great a depth as the more conspicuous response in the endodermis. This increase in cult to distinguish from those of the endodermis. Divisions occur progressively outward, so that by the sixth day the entire cortex over the major bundles may be proliferating (fig. 7). These divisions in the outer cortical parenchyma are also in various planes, and they appear as patches of meristematic activity (fig. 13A). Proliferation of the outer cortical parenchyma is greatest near
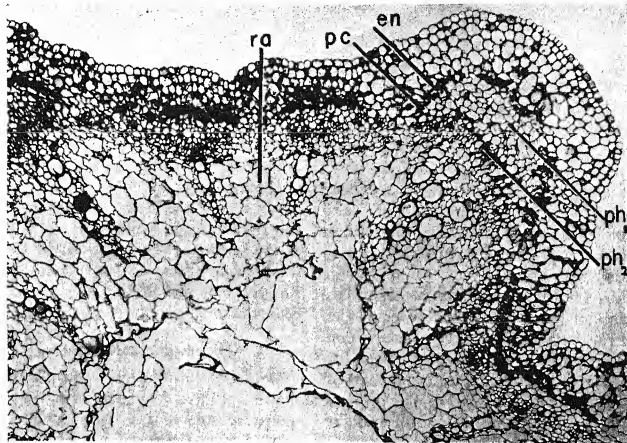
Fig. 1.—Transection of second internode of control stem, 1.5 mm. below cut surface, 15 days after application of lanolin: *en*, endodermis; *ph₁*, primary phloem; *ph₂*, secondary phloem; *ra*, ray. Note single layer of endodermis, thick-walled pericyclic fibers, wide cap of primary phloem parenchyma over large bundles, narrow zone of cambium, lignified vessels in secondary xylem, and inactive cells of rays. Disintegration of pith characteristic of older bean stems.

size is more marked in the inner cortex and is accompanied by increase in the meristematic condition of the cells. Divisions follow, so that by the fourth day cells adjacent to the endodermis have divided one or more times and in various planes (fig. 3). In older stems proliferation of the inner cortical parenchyma extends down to 5.0 mm., and derivatives of this proliferation become diffi- the cut surface and extends down about 1.0 mm. The derivatives principally mature as parenchyma, although strands of meristematic cells may persist, especially near the cut surface. Chloroplasts are found in the cortical tissues at the lower levels of response but are reduced in size or even disappear in proximity to the cut surface.

The endodermis is the first tissue to

react to treatment with phenylacetic acid, and the response reaches its greatest vertical depth in this tissue. Within 48 hours the cells enlarge radially, and there are divisions over the large bundles near the cut surface (fig. 2A). As the response extends down the stem, the cells usually enlarge (fig. 2B) and then divide. Unlike cortical parenchyma, these divisions may reach to within a few cells of the lowest level of response. By the fourth day this level is about 5.0 mm. from the cut surface and ultimately extends down to 8.0 or 9.0 mm. Proliferation in the endodermis is characterized by numerous tangential divisions (fig. 12), although divisions in other planes may occur. Because of this irregular pattern, distinction between the derivatives of endodermis and the inner cortical parenchyma may be lost, and the two proliferating tissues may form a conspicuous band of active derivatives capping the vascular bundles (fig. 3). In the more advanced stages of response many of these derivatives mature as reticulate tracheids. Scattered among these tracheids may be enlarged parenchymatous cells, groups of meristematic cells, or poorly organized vascular bundles (fig. 13A, B).

The pericycle is relatively inactive. Less than 0.5 mm. below the cut surface, pericyclic cells which were parenchymatous at the time of treatment are crushed by the adjacent proliferating cells. At slightly lower levels they may persist as parenchyma cells whose identity is quickly lost among other parenchyma. At 1.0 mm. or more below the treated surface, where the cell walls were already thickening, pericyclic cells mature as fibers and are readily identified (fig. 5).

The primary phloem parenchyma responds quickly, showing increase in cell size within 48 hours after treatment.

This enlargement finally reaches a depth almost as great as that of the endodermis. Response in this phloem tissue is similar to that of the inner cortical parenchyma, both in time of reaction and in type of proliferation. In more advanced stages primary phloem derivatives tend to mature as reticulate tracheids, phloem strands, or poorly organized bundles, although some meristematic areas and large parenchymatous cells may also be found (fig. 7). Secondary phloem increases in amount somewhat but shows little proliferation. By the fifth day patches of matured secondary phloem are pushed out, either by differentiation of more secondary phloem parenchyma or by enlargement of parenchyma already present (fig. 7).

The cambium is a highly sensitive tissue, showing increased activity by the second day. This results in a wide zone of undifferentiated cells (fig. 3), an effect which extends to nearly the same depth as the response in the endodermis.

Secondary xylem differentiated after treatment is in the form of tracheids. No vessels were formed in this secondary xylem (compare figs. 6B, 7, 12 with fig. 1). By the fifth day xylem tracheids are differentiated completely around the stem, except in some of the rays (fig. 7). Much more secondary xylem than secondary phloem is formed from cambial derivatives, and this is particularly evident near the cut surface (fig. 10).

Parenchyma of the ray is highly sensitive but diverse in its response. Ray cells flanking the phloem and the fascicular cambium are meristematic within 48 hours (fig. 2A), and by the fifth day an interfascicular cambium is established across many of the rays (fig. 6B). In the region exterior to this cambium, proliferation is marked. Near the cut surface narrow bands of meristematic cells (fig. 11) are often formed along the mar-
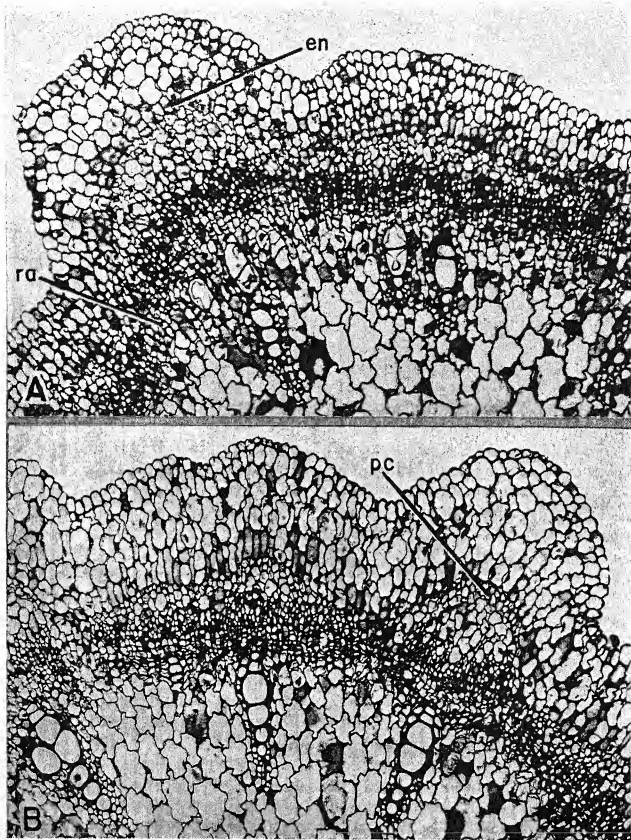
FIG. 2.—*A*, transection 2 days after treatment with 2% phenylacetic acid in lanolin, 0.5 mm. below cut surface, showing radial elongation and tangential divisions of endodermal cells. Cortical cells enlarged, inner ones becoming meristematic; increased activity in cambium resulting in wide band of undifferentiated cells; *ra* becoming meristematic. *B*, same stem, 2.5 mm. below cut surface. Endodermal cells enlarged but not divided; greater maturity of pericyclic fibers and secondary vascular elements.
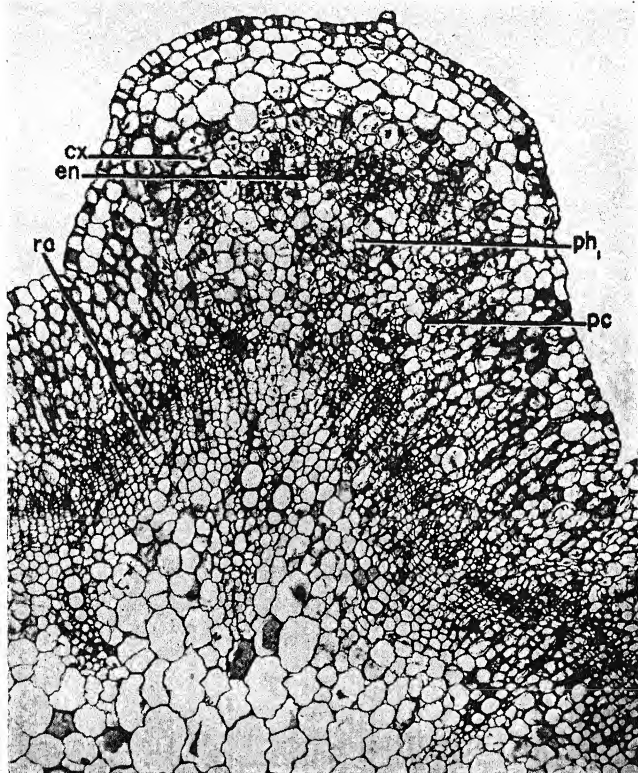
FIG. 3.—Transection 1.5 mm. below cut surface, 4 days after treatment: *cx*, cortical parenchyma. Detail of large bundle at level of greatest response showing many tangential divisions in endodermis; characteristic irregularity in plane of divisions in inner cortical parenchyma and primary phloem; pericyclic layer disorganized; wide zone of undifferentiated cells from cambium with many tracheids along inner margin; increased meristematic activity across rays.
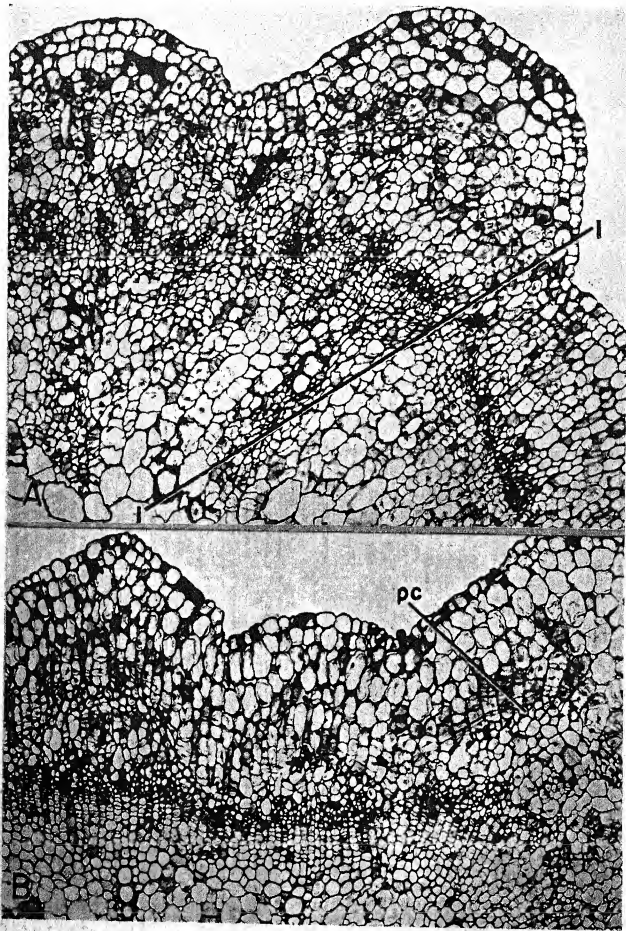
FIG. 4.—Same stem as fig. 3, 4 days after treatment. *A*, transection just below cut surface. Proliferation in rays and pith associated with development of tumor. Degree of response in inner cortical parenchyma, endodermis, phloem parenchyma, and cambium less at cut surface than at 1.5 mm. (fig. 3). Line 1 similar in structure to line 1 in fig. 5. *B*, transection 2 mm. below cut surface, showing less activity. Well-developed pericyclic fibers and greater maturation of other tissues.
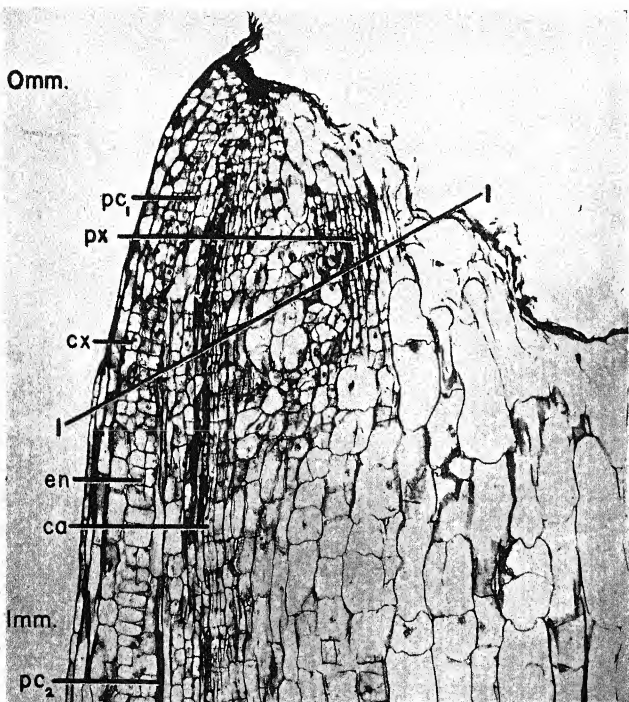
FIG. 5.—Longisection of decapitated stem 4 days after treatment: *px*, protoxylem; *ca*, cambium; O *mm.*, approximate level of cut surface as determined by severed vessels. Cells of cortical parenchyma enlarged and dividing tangentially. Pericyclic cells at upper levels (*pc₁*) crushed by adjacent tissues; at lower levels (*pc₂*) maturing as fibers. Cells of primary phloem parenchyma greatly enlarged and a few divided. Tracheids differentiating centrad to active cambium. Parenchyma adjacent to vascular areas meristematic; central pith inactive and collapsing at wounded surface. (Compare line 1 with line 1, fig. 4*A*.)
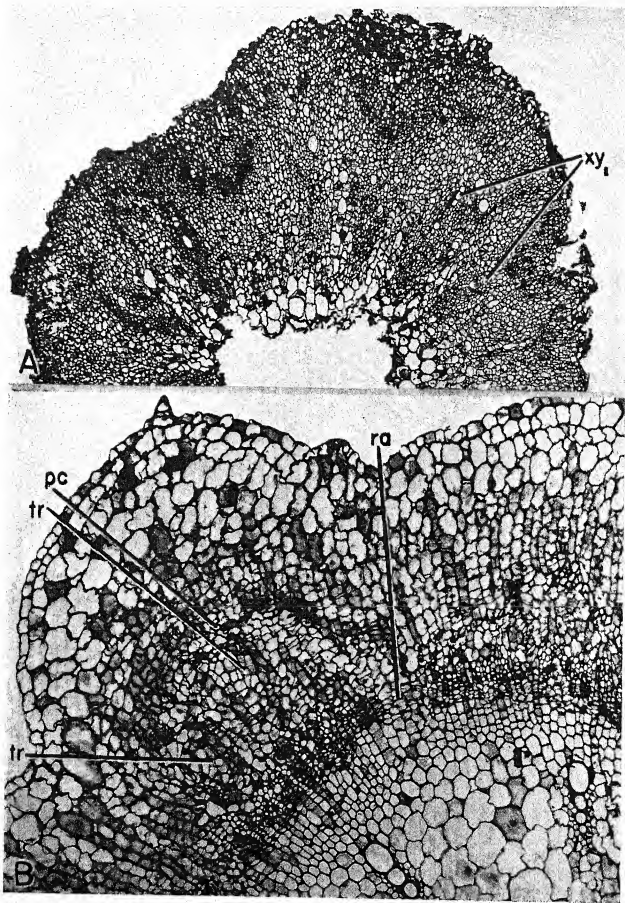
FIG. 6.—Transection 5 days after treatment. *A*, highly meristematic tissues at cut surface: *xy₁*, primary xylem. Proliferation mainly from phloem, cambium, young xylem elements, rays, and pith centrad to proto-xylem. *B*, 1.5 mm. below cut surface: *tr*, tracheid. Lessened effect at lower level indicated by absence of outer cortical and pith activity, less proliferation of endodermis and phloem. Note fewer tracheids matured from phloem derivatives.
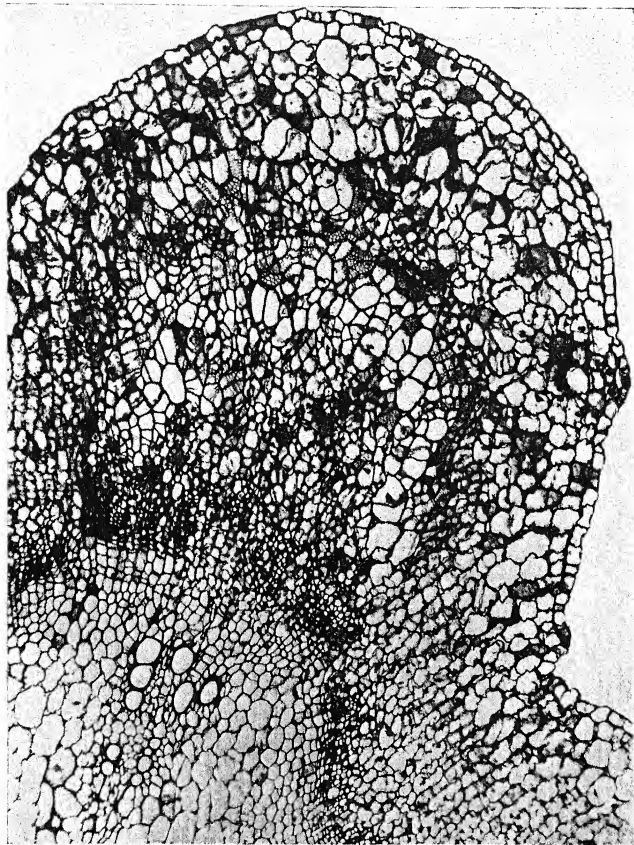
FIG. 7.—Same stem as fig. 6, 5 days after treatment, 1 mm. below cut surface, showing large bundle. Outer as well as inner cortical cells dividing in various planes. Many derivatives of inner cortical parenchyma, endodermis, and primary phloem matured as reticulate tracheids; other derivatives continuing meristematic activity, some forming strands of phloem or small, poorly organized vascular bundles. Note increase in secondary phloem (ph_2) and in ray tracheids.
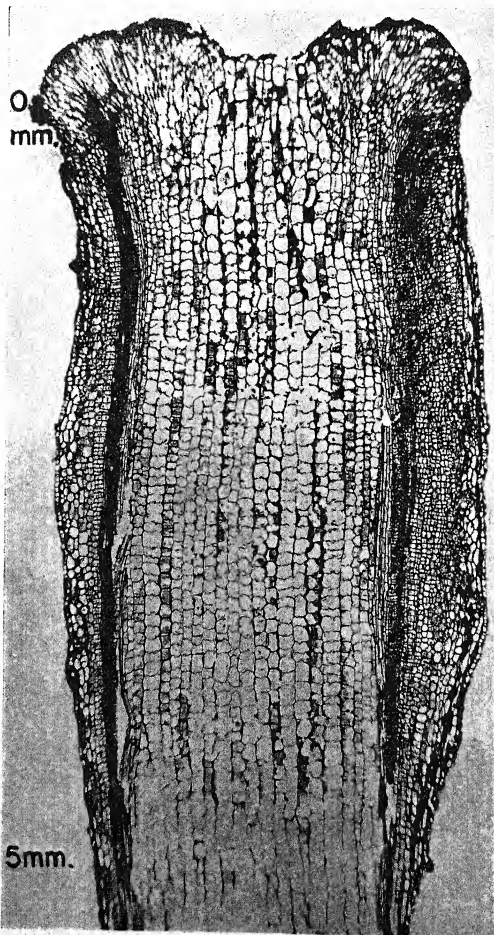
Fig. 8.—Longisection 6 days after treatment. Early stage in formation of tumor above original level of cut surface. Central pith and outermost stem tissues not contributing to tumor proliferation.
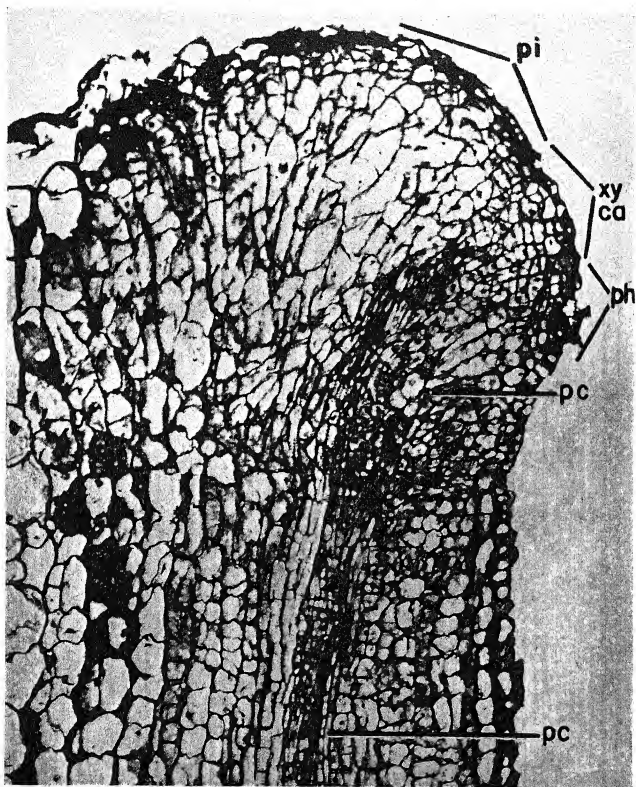
FIG. 9.—Detail of fig. 8: *pi*, pith; *xy*, xylem. At original level of cut surface, proliferation of phloem, cambium, young xylem elements, and pith and ray cells adjacent to xylem forming young tumor around collapsing central pith. Tissues of pericycle and cortex forced outward and not active in formation of tumor.

gins of the rays next to the phloem. Such strands connect the fascicular cambium and similar strands formed in the cortex and the phloem. At lower levels these cortical and phloem strands are differentiated into the poorly or-ganized vascular bundles already de-scribed. In older stems activity in the outer ray parenchyma and in the inter-fascicular cambium ceases upon the maturation of many cells as tracheids (fig. 13A). Ray parenchyma cells flank-
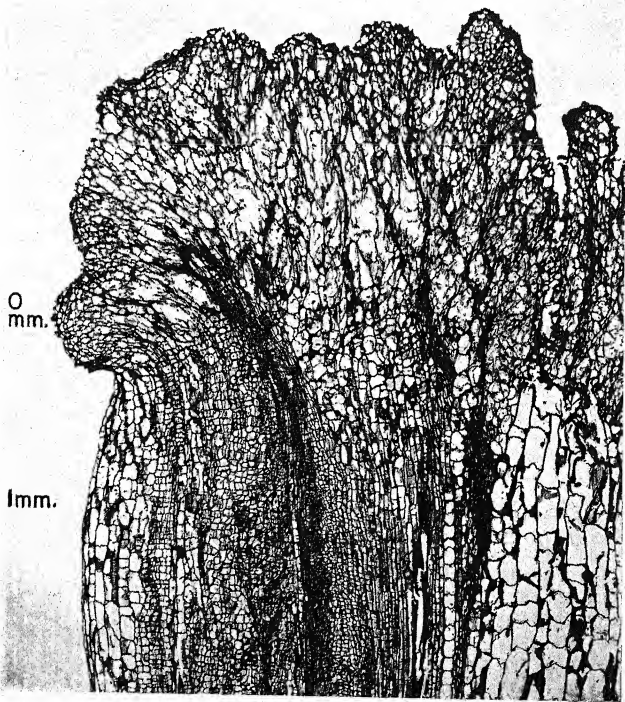

Fig. 10.—Longisection of stem 10 days after treatment, section through vascular bundle. Peripheral areas of tumor composed of compact meristematic tissue; internal region large parenchyma cells interlaced with small folds of collapsed cells. Conspicuous strands in tumor small vascular bundles differentiating from active pith adjoining primary xylem and from cambium and its adjacent derivatives. At lower levels note tracheids in cortex, endodermis, primary phloem, and secondary xylem.

ing the secondary xylem may divide and then differentiate as a region of reticulate tracheids across the rays and adjacent to the secondary xylem (fig. 7). Proliferation of the innermost ray parenchyma is similar to that of pith parenchyma, and the two may be described together.

larization of the tumor (fig. 10). The central pith area remains inactive and tends to disintegrate (fig. 14).

The pattern of proliferation in the apical tumor is modified by the nature of the response lower in the stem. Development of the tumor is clearly evident by the sixth day (figs. 8, 9) in the
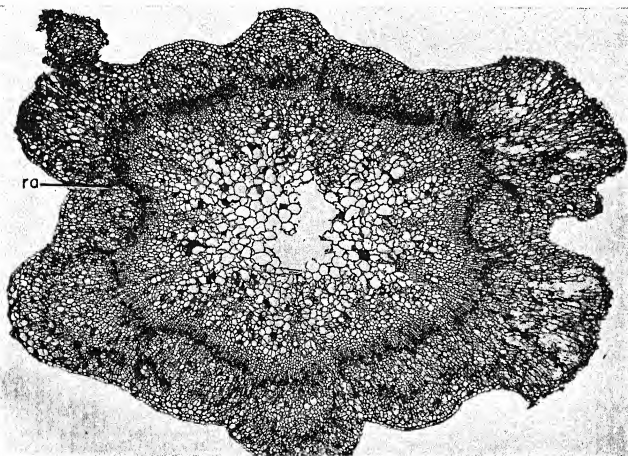

FIG. 11.—Transection of stem about 0.5 mm. below cut surface, 10 days after treatment. Irregular proliferation at periphery mainly primary phloem parenchyma associated with tumor. Pith proliferated centrad to primary xylem. Meristematic strands in rays (ra).

The peripheral pith is highly responsive at the cut surface and down to about 1.5 mm. This activity is associated with proliferation in the formation of the tumor. Divisions occur in the parenchyma centrad to the protoxylem points, and in the rays between the bundles (figs. 4A, 6A). In older stages small vascular bundles and many reticulate tracheids are differentiated. These new bundles may be directly related to vascu-

upward proliferation of the pith, ray, young xylem, cambium, and phloem—tissues which are already active at lower levels in response to the phenylacetic acid. Although tissues from the pericycle outward also proliferate in response to the growth substance, they do not take part in the formation of the tumor. Instead they are deflected outward by the growth of the tumor above them. Older tumors show a surface layer of suberized

cells (figs. 10, 14). Beneath this is a relatively compact mass of cells which may be meristematic. The internal and greater mass of the tumor consists of comparatively large, thin-walled parenchymatous cells interlaced with folds of smaller, collapsed parenchyma. A few

also occurs. The central mass of the tumor is derived from proliferated pith and ray parenchyma, with outer portions from young xylem, cambium, and phloem. The last participates especially in forming the peripheral lobes extending down around the stem.
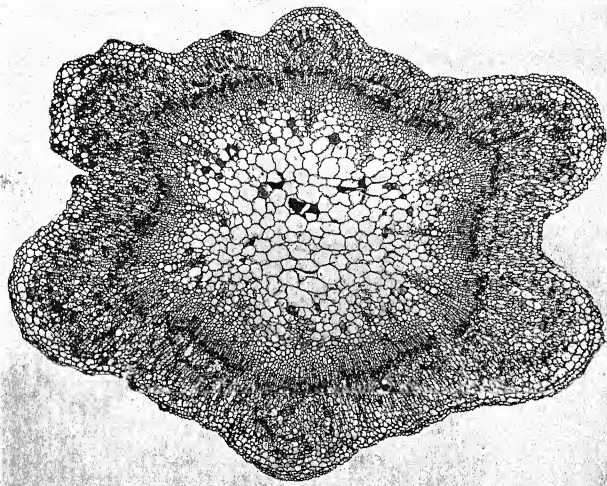

Fig. 12.—Transection of same stem as fig. 11, 10 days after treatment, 1.5 mm. below cut surface. Pattern of response below tumor; over larger bundles marked proliferation of cortex, endodermis, and phloem; over smaller bundles mainly proliferation of endodermis. Vessels lacking in wide band of secondary xylem tracheids.

poorly organized vascular bundles arise from the stem below, anastomose in a simple pattern, and branch outward to the periphery. Some of these bundles are directly connected with those formed in the proliferated pith adjacent to the primary xylem; others seem to originate in the region of the young vascular elements. Independent differentiation of vascular elements and wound tracheids

Initiation of root primordia was infrequent in the material studied. By the fourth or fifth day near the cut surface small areas of the endodermal and cortical derivatives between the bundles may show intense meristematic activity. This activity extends along the ray to the cambial zone. By the sixth day the organization of a root primordium is evident (fig. 16), with the central cylinder
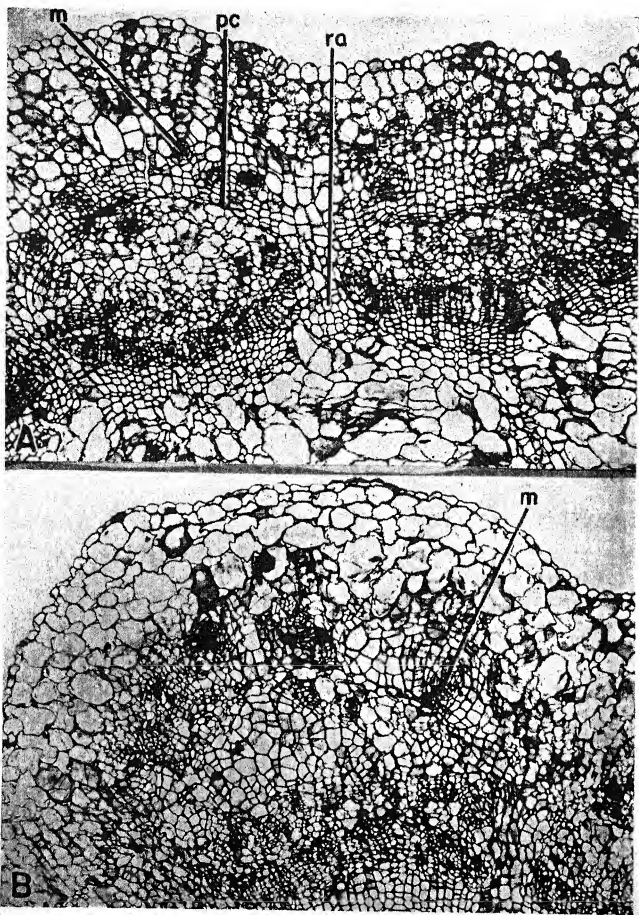
FIG. 13.—A, transection of stem 10 days after treatment, 1 mm. below surface: m, meristematic cells. Detail showing differentiation of tracheids centrad to cambium, outward along rays, and arching over phloem in inner cortical derivatives. Small patches of meristematic cells (m) in outer cortex and within areas of differentiated tracheids. B, transection of stem, 3.0 mm. below cut surface, 14 days after treatment. Greater differentiation of tracheids and continued proliferation in meristematic patches, those surrounded by tracheids (m) showing centers of crushed cells. Outer cortex not proliferated at this level.

formed from ray and adjacent phloem derivatives and the capping tissues apparently from the endodermis and inner cortical parenchyma. The organization of the primordium was not generally well defined, and no further development was found in older material.

Discussion

Phenylacetic acid belongs to the group of growth-regulating substances which cause localized responses in the plant. Among the general features of such local responses in the bean, the following appear to be characteristic for phenylacetic
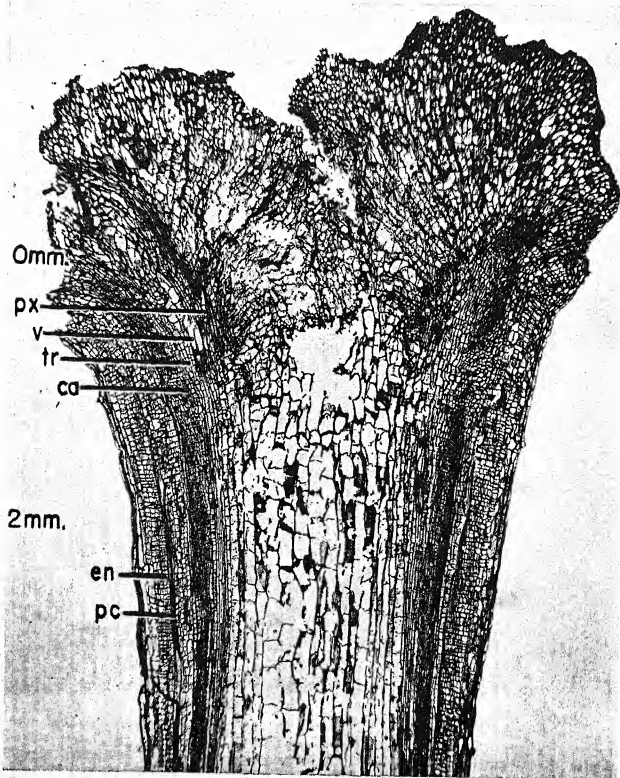

FIG. 14.—Longisection of small but mature apical tumor 4 mm. across, 20 days after treatment. Tumor composed mainly of proliferated xylem, ray, and pith tissues. Central pith completely inactive and breaking down. Below tumor, endodermis and primary phloem proliferated and wide zone of cambium evident.

acid as applied in this experiment: (a) high degree of response in the cortical tissues; (b) frequent differentiation of reticulate tracheids in the maturation of proliferated tissues; (c) secondary xylem differentiated after treatment composed of tracheids but no vessels; (d) limited vascularization of proliferated tissues, including the apical tumor; (e) proliferation of the apical tumor largely from xylem, pith, and ray derivatives; (f) infrequent occurrence of root primordia.

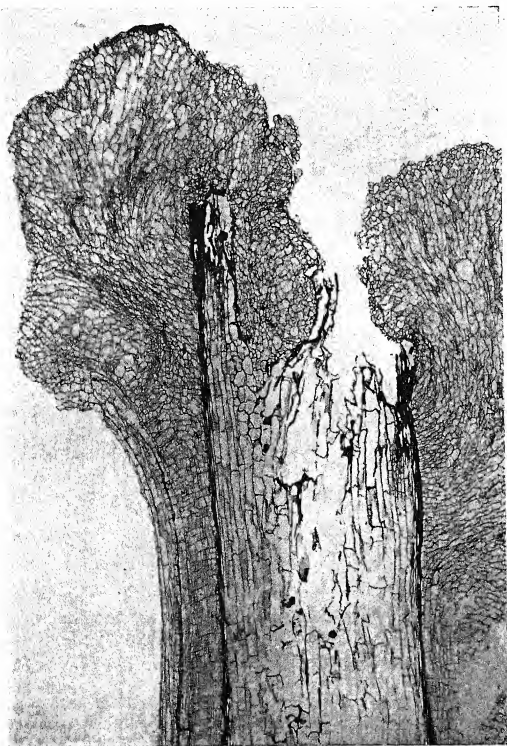This paper corroborates in general the more limited description of BLUM (1) for

FIG. 15.—Longisection of apical tumor of control plant with cut surface untreated, 15 days after decapitation. Tumor developed almost entirely from proliferated phloem. Xylem, ray, and pith tissues inactive and many cells disintegrating. No proliferation of tissues in stem below tumor; cf. fig. 14. Fig. 15 taken from previous report (6).

the response of sunflower to phenylacetic acid. However, these results are not exactly comparable because of the difference in concentrations used, 0.2% for the sunflower and 2.0% for the bean. In a comparison of responses to five different growth substances he lists

not been described for the bean. Perhaps the higher potential of tumor proliferation for the bean may account for some of these differences.

A comparison of various growth substances applied to bean reveals that phenylacetic acid is markedly similar to
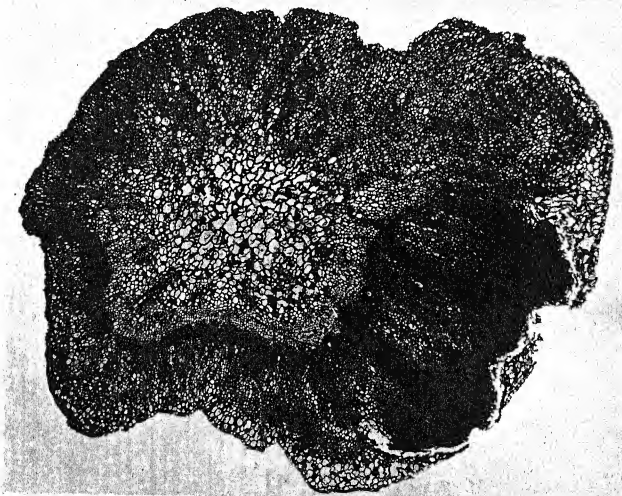

FIG. 16.—Transection immediately below cut surface 6 days after treatment, showing character of root development from ray and adjacent phloem, with capping tissues probably from endodermal and cortical derivatives. Root primordia similar to those initiated by treatment with indoleacetic acid, but occurrence infrequent.

the outward divergence of cambium between the vascular bundles as the distinguishing characteristic for phenylacetic acid. In the bean, interfascicular cambium may extend directly across the ray or diverge outward slightly (fig. 6B), but this is never a consistent and striking feature. BLUM also describes the formation of a horizontal cambium across the cut end. Such a cambium has

corn-pollen extract (8) in the response it produces. This is particularly evident in the proliferation and tracheid differentiation of the cortical tissues and the outer rays. With extract of corn pollen the outer cortical parenchyma exhibits greater activity, but tissues from the phloem inward are more active with phenylacetic acid. Certain of the other growth-regulating substances induce re-

sponses unlike those for phenylacetic acid. Among characteristic differences are: the high degree of proliferation, vascularization, and frequency of root formation resulting from the application of indoleacetic, indolebutyric, and naphthaleneacetic acids; the great activity of the xylem in response to tetrahydrofurfuryl butyrate; the heavy wall-thickening and increase in secondary xylem with naphthalene acetamide.

Other workers (10, 11) have reported induction of root primordia upon application of phenylacetic acid. No adventitious roots were reported by BLUM (1), and in the present experiment only a few cases of initiation of root primordia were noted. Differences in concentration of the acid and in the humidity (well below saturation at all times during this experiment) may have influenced the results. At least no definite conclusion can be drawn from this histological study.

FLINT and MORELAND (2) report that humidity is also a factor in the formation of the bean tumor. They suggest that under some conditions tumor development considered as a response to an applied growth substance can be obscured by the action of natural wound hormones. On the basis of gross observations, distinction cannot always be made between the tumors produced by natural wound hormones and those induced by applied growth substances. Histological examination, however, as represented in figures 14 and 15, shows that there are significant tissue differences in tumor proliferation between an untreated decapitated stem and one treated with phenylacetic acid.

Summary

1. Young bean plants, *Phaseolus vulgaris* var. Red Kidney, were decapitated at the second internode, and a 2% mixture of phenylacetic acid in lanolin was applied to the cut surface.

2. Within 48 hours the stem tip lightens in color and begins to enlarge, approximately doubling in diameter by the sixth day. Formation of an apical tumor is evident by the fifth day, reaching maximum growth on the eleventh to fifteenth days, after which growth gradually decreases, and many stems are dead by the thirtieth day. The tumors are flat-topped, somewhat tuberculate over the surface, and vary in size up to 9 mm.

3. Histological reactions below the cut surface consist of marked proliferation of inner cortical parenchyma, endodermis, and primary phloem parenchyma. Less active are the outer cortical parenchyma, the rays, and the peripheral pith. The derivatives may mature as tracheids or parenchyma, continue as patches of meristematic tissue, or infrequently differentiate as small vascular bundles. After treatment there is increased activity of the cambium, its secondary xylem derivatives differentiating entirely as tracheids. Response is slight in the pericycle and secondary phloem; the epidermis and the central pith are inactive.

4. Response at the cut surface results in the upward proliferation of a parenchymatous tumor. The central mass of the tumor is derived from proliferated pith and ray parenchyma, with outer portions from young xylem, cambium, and phloem. Vascularization of the tumor is slight. The small, branching strands connect with the vascular bundles of the stem, or with bundles differentiated in the pith. Centripetal growth of the tumor fills in the cavity formed by collapse of the central pith.

5. Root primordia are infrequent.

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EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE WATER RELATIONS, THE ACCUMULATION AND DISTRIBUTION OF SOLID MATTER, AND THE RESPIRATION OF BEAN PLANTS

JAMES W. BROWN[1]

Introduction

Attention has recently been directed toward the use of growth-regulating chemicals for killing noxious weeds (1, 2, 3, 5, 6). The ultimate toxic effects these substances have, when applied in relatively large amounts, involve prolonged physiological responses associated with death of the plants. Experiments have shown that sugars and carbohydrate reserves in the form of starch and dextrin were rapidly depleted in annual morning-glory plants that were sprayed with 2,4-dichlorophenoxyacetic acid (4), a response which occurred within 21 days after treatment.

The present experiments were undertaken for the purpose of studying further the responses that may be associated with the death of plants that have been treated with toxic amounts of 2,4-dichlorophenoxyacetic acid. Bean (*Phaseolus vulgaris*) seedlings were sprayed with various amounts of the acid, and the effects on the plants determined for (*a*) the water absorption, transpiration, and water content,, (*b*) the accumulation and distribution of solid matter, and (*c*) respiration.

Experimental data

TRANSPIRATION

METHODS.—Bean seeds of the Black Valentine variety, the Asgrow strain,[2] were planted in 3-inch clay pots con-

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[2] Seeds furnished by Associated Seed Growers, Incorporated.

taining composted soil and grown under greenhouse conditions. Each pot contained one plant. The second day after emergence, fifty seedlings were selected for general uniformity. At this stage the plants were 5–7 inches tall and the primary leaves had expanded, but the second internodes had not yet elongated and the trifoliate leaves were still within the terminal buds.

Twenty of the pots were sealed in envelopes made of moisture-proof cellulose acetate sheeting (fig. 1). In constructing the envelopes, a wooden mold the shape of a 3-inch clay pot was used as a pattern and the sheeting sealed along the side and across the bottom by means of a hot iron. One of the potted plants was inserted into each envelope and its open end sealed, allowing the stem to protrude through a closely fitted hole. A small slit in the sheeting, kept closed by means of Scotch tape when not in use, permitted watering the plant.

The twenty plants inclosed in the envelopes were weighed, then ten of them were sprayed with an aqueous mixture containing 1000 p.p.m. (0.1%) of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500, while the remaining ten were left unsprayed. Paper covers were placed around the stems of the plants to shade the pots from direct sunlight and thus prevent the temperature within the envelopes from becoming unusually high. All twenty plants were then arranged in alternate rows on a greenhouse bench. Weighings were made on successive days to determine the amount of water lost, and, when necessary, water was added to the pots to maintain an optimum moisture supply.

In order to determine the effect of treatment on the water content of treated and untreated plants comparable with those inclosed in the envelopes, ten of

the remaining thirty plants were sprayed with the acid mixture and twenty left unsprayed. Ten of the unsprayed plants were harvested immediately to determine their water content at the beginning of the experiment, and the remaining ten unsprayed plants were harvested at the end of the experimental 5-day period for similar determination. In preliminary experiments it was found

FIG. 1.—Bean seedlings growing in pots sealed in moisture-proof cellophane envelopes. *A*, unsprayed plant. *B*, plant sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500. Twenty-four hours after treatment.

that, although the second internodes of plants with their roots inclosed in the envelopes were slightly shorter than those of plants grown in open pots, their over-all growth was not significantly affected.

RESULTS.—Within 1 hour following treatment, the sprayed seedlings showed marked epinastic responses and stem bending, which became more severe during the next 2 or 3 days. At the end of 4 days the leaves and buds showed no apparent increase in size; the primary

leaves were permanently wilted at the end of 5 days; and at the end of 7 days the plants were dead. Necrotic areas and vigorous fungus growth appeared on most stems at the soil surface. Growth of the fungi occurred throughout a region of the stem tissue which extended 1–3 cm. above the surface of the soil. Symptoms of the presence of these organisms, which may have contributed to the ultimate death of the plants, were usually not apparent until after the plants seemed to be beyond recovery. On the other hand, untreated plants grew
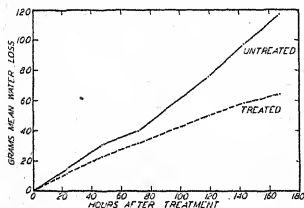

FIG. 2.—Transpiration of bean plants compared with that of others sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500.

vigorously during the week following treatment and their stems showed no similar symptoms.

The rate of water loss from untreated plants was rather constant for a period of 7 days following treatment (fig. 2), while the treated plants transpired at a gradually decreasing rate. Treated plants lost 21% less water during the first 23 hours than did the untreated ones, a difference statistically significant at the 1% level. At the end of 7 days the accumulated water loss of the treated plants was 45% less than that of the unsprayed ones. Since water loss was determined by weighing together pot, soil, plant, and envelope, the observations included the

weight of any photosynthate and water which accumulated in the plants during the experiment. This accumulation was negligible, however, since the combined weight increase due to the accumulation of solid matter and water during the 7 days following treatment did not amount to more than 2% of the total water loss. The transpiration data were therefore not greatly affected by these factors.

An estimation of the water uptake of the plants was obtained by first subtracting the average amount of water in them at the beginning of the experiment from that in them 5 days later, to ascertain the increase. This amount was then added to the weight of water transpired during the 5-day period following treatment and the result designated as the total amount of water absorbed. Although the treated plants lost 33.9% less water than did the untreated ones, the percentage of absorbed water transpired was the same (98.6%) for both treated and untreated plants (table 1).

ACCUMULATION AND DISTRIBUTION OF WATER AND SOLID MATERIALS

METHODS.—Bean seedlings (Black Valentine) were grown from seed as previously described. Sixty plants were selected for general uniformity to be used in determining the effect of applying the various amounts of 2,4-dichlorophenoxyacetic acid. These plants were at the same stage of development as those used in the previous experiment. The plants were divided into six groups of equal number. One group was harvested at the time of treatment, and another untreated group was harvested at the end of the experiment, so as to determine the increase in weight of the unsprayed plants during this interval and thus obtain a basis for comparison of the synthesis of solid matter and accumulation of water

in the treated ones. The remaining four groups were used for testing the effects of four levels of concentration of the acid when applied as a spray. The concentrations were 25, 50, 250, and 1000 p.p.m., and each acid mixture contained 0.6% of Carbowax 1500.

All plants were harvested at the end of 4 days to determine the degree of response of those sprayed with the lower concentrations as compared with that of the plants treated with the 1000 p.p.m. mixture.

At the time of harvest, the plants individually were sectioned into hypocotyls, first internodes, petioles, leaves, and terminal buds (all parts above the second node). Fresh weights of these parts for each plant were obtained immediately after they were detached. They were then dried at 80° C. for 24 hours and the dry weights recorded.

RESULTS.—All sprayed plants showed characteristic epinastic responses and stem curvatures, as previously described. At the end of 4 days the plants sprayed with the 1000 p.p.m. solution were markedly distorted, and the leaf blades became permanently wilted during the fifth day. All treated plants grew at a slower rate than did untreated ones during a 4-day period following treatment, since the sprayed plants gained 48–66% in fresh weight, while the untreated ones gained 88% during this time (table 2). Generally, the higher concentrations had successively greater retarding effects on increase in weight. During the test the unsprayed plants became slightly less succulent than they were at the beginning of the experiment. Solid matter in the unsprayed plants increased 164%, while water increased only 85%. The amount of solid matter accumulated by sprayed plants during the 4-day period (49–84%) was far less than

that accumulated by the unsprayed ones, the more concentrated treatments being associated with lower dry-weight accumulation.

The effect of various amounts of 2,4-dichlorophenoxyacetic acid on the growth of leafy parts of the plants (expanded leaf blades and buds) was determined on the basis of (a) the amount of solid matter they gained following treatment, (b) the amount of water they contained, and (c) the rate of change in area of leaf surface occurring following

TABLE 1

TRANSPIRATION AND ESTIMATED WATER UP-TAKE OF SEEDLING BEAN PLANTS SPRAYED WITH AQUEOUS MIXTURE OF 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500. MEAN WATER UPTAKE AND TRANSPIRATION OF TEN PLANTS DURING 5 DAYS FOLLOWING TREATMENT EXPRESSED IN GRAMS

	Un-treated	Treated	Differ-ence (%)
Transpiration............	75.419	49.875	33.9
Estimated water uptake.	76.500	50.584	33.9
Percentage of uptake transpired...........	98.6	98.6	......

treatment. The accumulation of solid matter in the leaf blades and buds was significantly reduced as the result of the application of mixtures containing 25 p.p.m. or more of the acid (table 3). This effect of inhibiting leaf growth was very marked at the end of 4 days, since 74% less solid matter accumulated in the leaves of plants sprayed with a mixture containing 25 p.p.m. of the acid than in the leaves of unsprayed plants, and the reduction was 91% in the case of those sprayed with 1000 p.p.m. The amount of water that accumulated on an absolute basis in the buds and leaf blades of treated plants was 65–91% less than that

in comparable parts of untreated plants during the 4-day period (table 3). In contrast, the water content of the buds and leaf blades of the treated plants, expressed as a percentage of the final fresh weight, was slightly greater (89.7–90.4%) than was that of comparable parts of unsprayed plants (88.8%).

Leaf area of the plants sprayed with 1000 p.p.m. concentration increased 30% during the 48-hour period immediately following treatment, while that of the untreated ones increased 55% (table 4). The rate of leaf expansion by the sprayed plants became progressively less, and at the end of 5 days the leaves were permanently wilted and their area averaged 9% less than at the

TABLE 2

RESPONSE OF SEEDLING BEAN PLANTS 4 DAYS AFTER SPRAYING WITH AQUEOUS MIXTURES CONTAINING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. ALL WEIGHT RECORDS ARE RELATIVE, BASED ON DETERMINATIONS AT BEGINNING OF EXPERIMENT AS 100

Treatment (p.p.m.)	Hypocotyls	First internodes	Petioles	Primary leaves	Terminal buds	Total aerial part
			Fresh weight			
0...............	93	172	238	234	3025	188
25...............	144	199	143	153	674	157
50...............	158	236	156	145	895	166
250...............	167	253	160	107	619	155
1000...............	124	217	327	111	515	148
			Solid matter			
0...............	182	291	303	251	2908	264
25...............	192	267	165	148	462	176
50...............	217	295	163	143	515	184
250...............	196	281	144	105	300	152
1000...............	137	240	384	115	308	149
			Water content			
0...............	87	166	236	232	3043	185
25...............	141	195	141	153	707	155
50...............	155	231	156	145	954	164
250...............	166	252	160	107	668	155
1000...............	121	215	324	110	546	148
			Percentage moisture			
0 (Initial).......	93.8	94.7	94.6	89.9	86.6	92.4
0 (Final).......	87.9	91.0	93.7	89.2	87.1	90.9
25...............	91.8	92.9	93.8	90.2	90.8	91.5
50...............	91.6	93.4	94.4	90.0	92.3	91.6
250...............	92.8	94.1	95.1	90.1	93.5	92.5
1000...............	91.9	94.1	93.7	89.5	91.8	92.3

beginning of the experiment. The leaf area of untreated plants increased 94% during the same period.

In contrast with the inhibitory effect on leaf growth, the amount of solid matter accumulated in the hypocotyls of plants treated with mixtures containing 25, 50, or 250 p.p.m. of the acid was 13–43% greater than in the hypocotyls of unsprayed plants (table 5). With the application of an extremely high concentration (1000 p.p.m.) the hypocotyls of sprayed plants failed to accumulate more

TABLE 3

INCREASE DURING 4 DAYS IN DRY WEIGHT AND WATER CONTENT OF LEAVES AND BUDS OF SEEDLING BEAN PLANTS SPRAYED WITH AQUEOUS MIXTURES CONTAINING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RECORDS OF INCREASES ARE RELATIVE, BASED ON INCREASE IN UNTREATED PLANTS AS 100

Concentration (p.p.m.)	Dry weight	Absolute water content
0.	100	100
25.	26	35
50.	25	33
250.	4	9
1000.	9	10

solid matter than did those of the unsprayed ones. The over-all accumulation of solid matter in the above-ground portion of the plants, however, was greatly reduced (49–70%) by the application of mixtures containing 25 p.p.m. or more.

With respect to water content, the hypocotyls of treated plants gained 21–66% during the 4-day period immediately following treatment, while the untreated plants lost 13% of the amount they contained at the beginning of the experiment (table 2). Although treatment with 2,4-dichlorophenoxyacetic acid increased the water content of the hypocotyls, the amounts of moisture on

an absolute basis in the entire aboveground part of sprayed plants at the time of harvest were less than for unsprayed ones (table 2). In contrast, the water content expressed as a percentage of their total fresh weight was slightly

TABLE 4

RELATIVE LEAF AREAS OF SEEDLING BEAN PLANTS SPRAYED WITH 1000 P.P.M. CONCENTRATION OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. LEAF AREA AT TIME OF TREATMENT DESIGNATED AS 100

Days after treatment	Untreated	Treated
0.	100	100
2.	155	130
5.	194	91

TABLE 5

INCREASE DURING 4 DAYS IN DRY WEIGHT OF HYPOCOTYLS AND OF ENTIRE ABOVE-GROUND PARTS OF SEEDLING BEAN PLANTS SPRAYED WITH CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RECORDS OF INCREASE ARE RELATIVE, BASED ON INCREASE IN UNTREATED PLANTS AS 100

Concentration (p.p.m.)	Hypocotyls	Above-ground parts
0.	100	100
25.	113	46
50.	143	51
250.	118	32
1000.	45	30

greater in the treated plants (91.5–92.5%) than in the unsprayed ones (90.9%), as shown in table 2. The petioles of unsprayed plants tripled their dry matter and more than doubled their weight of water in the 4 days. The 25, 50, and 250 p.p.m. treatments, however, retarded increase in water content of petioles to only 41–60% above the initial control. The striking point is that for petioles the 1000 p.p.m. treatment gave

increases in weight of solids, water content, and fresh weight far beyond the untreated final control. The petiole was the only part of the plant in which the 1000 p.p.m. treatment induced an accumulation of dry matter in excess of the final control. The first three treatments retarded solid matter accumulation in petioles and percentage of dry

scribed. For each of the respiration experiments approximately 400 seedlings were selected for general uniformity after the plants had attained a height of 6–8 inches (fig. 3A). Half the seedlings were sprayed between 10:30 and 11:30 A.M. with an aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500, and the re-
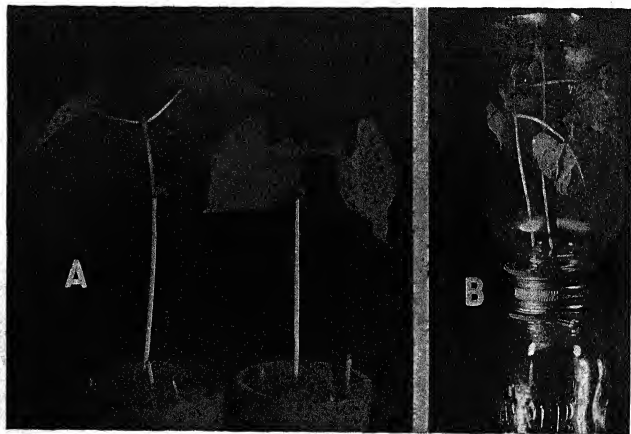

Fig. 3.—A, 1 hour after treatment: (left), untreated bean plant; (right), plant sprayed with aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. B, respiration chamber containing untreated seedlings.

matter progressively as their concentration increased. At the same time, water and total fresh weight were less progressively retarded. The marked increase in both water and solids in the most severe treatment resulted in an ultimate moisture percentage equal to that of the control petioles.

RESPIRATION

METHODS.—Black Valentine bean seedlings were grown as previously de-

mainder were left unsprayed and used as controls.

Carbon-dioxide output was used as a measure of respiratory activity in three separate experiments. In the first experiment, a temperature of 65° F. was maintained during the period that the CO_2 output was measured. In the second experiment it was 65° F. for 60% of the period and 75° F. for 40%. Plants in the third experiment were exposed to a temperature of 75° F. during the period that

their CO_2 output was measured. Plants
for all experiments were grown at green-
house temperatures of $70°–75°$ F., except
during the period of respiratory measure-
ment.

At 2:30 P.M. of each day on which
respiratory measurements were made,
thirty treated and thirty untreated
plants were taken from the pots and the
soil washed from their roots. Each group
was then divided into ten lots of three
plants, except that in the case of deter-
minations made at $75°$ F. each final
group consisted of two plants. Thus, in
each run the CO_2 output of ten groups of
treated and of ten groups of untreated
plants was measured simultaneously. At
the same time two additional chambers,
which did not contain plants, were used
as blanks.

Each respiratory chamber consisted
of a 1-quart and a 2-quart fruit jar con-
nected with a metal screw joint, thus
providing a closed system of 3-quart
capacity (fig. $3B$).[3] Aliquots of 0.05N
$Ba(OH)_2$ were delivered into the smaller
jars, which were then closed. The plants
were set upright in 50-ml. beakers which
had been filled with distilled water so
that the roots were completely immersed.
The beakers were suspended in the lower
jars above the $Ba(OH)_2$ solution by
means of copper wire attached to the
metal screw joints. The large jars were
inverted over the upper part of the plants
and the two jars joined, the joints tight-
ened, and the time recorded. After all

[3] This volume contained enough air to prevent
oxygen from becoming a limiting factor in the respi-
ration of the inclosed plants.

FIG. 4.—Bean plants following (right), spray
treatment with aqueous mixture containing 0.1% of
2,4-dichlorophenoxyacetic acid and 0.6% Carbowax
1500. Untreated plants on left. A, 1 hour; B, 1 day;
C, 5 days.
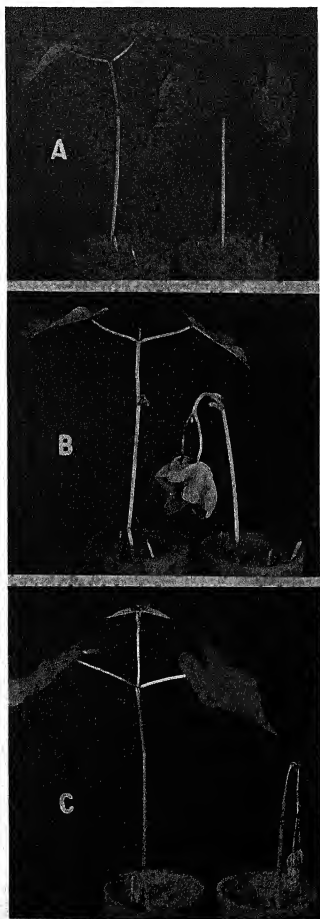
samples had been placed in chambers, they were moved to a temperature-controlled darkroom where they remained for 17–19 hours. After this period the time was recorded, the chambers opened, the water from around the roots poured into the alkali, the smaller jars closed again, agitated, and allowed to stand for 1 hour prior to titrating with 0.0535N HCl. The data for CO_2 output were computed on both a per unit of dry-weight basis and a per plant basis. The plants were dried for 30 minutes at 100° C. and then
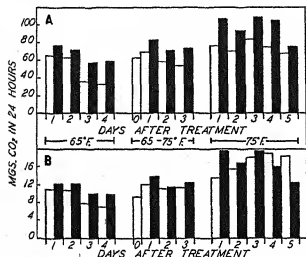

FIG. 5.—Respiration of bean seedlings on successive days following treatment with aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (solid bars) and of comparable untreated plants (open bars), computed (A) on basis of milligrams of CO_2 per gram of dry weight and (B) on per-plant basis; for temperatures of 65°, 65°–75°, and 75° F.

at 80° C. for 24 hours. They were weighed to the nearest milligram.

In a supplementary test, the CO_2 output of annual wild morning-glory plants (*Ipomoea lacunosa* L.) was measured. The plants were grown from seed in a greenhouse. After they reached the flowering stage, approximately 150 plants were sprayed with a 2,4-dichlorophenoxyacetic-acid mixture like that used for the bean seedlings and 150 of them were left unsprayed as controls. Four

days after treatment, five samples consisting of representative plants were taken from each group for measurement of CO_2 output. The number of plants in each sample varied from three to six. They were handled in the same manner as the bean seedlings and inclosed in glass jars. Their CO_2 output was measured during 2 hours of darkness at 75° F., after which they were dried and weighed as previously described.

RESULTS.—The sprayed seedling bean plants showed a bending response within 1 hour after treatment, and at the end of the fourth day following treatment they appeared to be beyond recovery, since their leaves were permanently wilted. By the fifth day collapsed stem tissue was evident in about 40% of the plants (fig. 4). It was noted on the fifth day of the experiment at 75° F. that actively growing molds and bacteria were present on 50% of the plant samples.

At all temperatures used, the production of CO_2 by sprayed plants was significantly greater than that of unsprayed ones during a period of 24 hours immediately after treatment (table 6; fig. 5). When calculated on a dry-weight basis, the CO_2 production remained significantly higher for the sprayed plants at all temperature levels for a period of 4 days. On a per plant basis, however, there was no significant difference on the second day after treatment between the amounts of CO_2 given off by treated and untreated plants at any of the temperatures. At the two lower temperatures subsequent measurements showed that the treated plants were once more respiring at a significantly greater rate than the untreated ones. The rate of CO_2 production (dry-weight basis) of sprayed morning-glory plants was significantly greater, by 80.6%, than that of unsprayed ones when measured

for a 2-hour period during the fourth day after treatment.

Discussion

Water uptake of bean seedlings was not immediately limited by spraying them with 2,4-dichlorophenoxyacetic acid, since the plants continued to transpire and their leaves did not wilt until 5 days after treatment, although the rate of transpiration was definitely diminished. This depression in rate of water loss was associated with marked curling of the leaves, marked decrease in the rate of leaf expansion, decrease in the rate of accumulation of water and solid materials within them, and an ultimate wilting of the leaves. The percentage of water uptake that was transpired was the same in both sprayed and unsprayed plants. It is apparent that the toxic effects of the acid were not associated with an excessive rate of water loss in proportion to the amount absorbed by the plants.

Application of the acid to bean seedlings had a marked effect upon the accumulation of water in the various parts of the plants, the rate of accumulation being greatly depressed in leaf tissues and accelerated in the stem tissues. The depression of leaf growth resulting from treatment cannot be accounted for on the basis of lack of water in the leaf tissues, however, since treated leaves did not contain less water on a percentage basis than did the untreated ones. Although the rate of accumulation of moisture in the stem tissues was greatly accelerated by the treatment, any relationship between this response and the toxicity of the acid is not clear. However, the increased succulence of the lower stem may have offered conditions favorable for the entrance and penetration of pathogens or other fungi, the indications

of which did not appear until after the plants were beyond recovery from the toxic effects of the treatment.

TABLE 6

PROGRESS OF CO_2 PRODUCTION AND DRY-WEIGHT CHANGES ON SUCCESSIVE DAYS FOLLOWING TREATMENT OF BEAN PLANTS WITH AQUEOUS SPRAY MIXTURE CONTAINING 0.1% OF 2,4-DI-CHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RESULTS EXPRESSED ON BASIS OF 100 FOR UNTREATED CONTROL PLANTS

DAYS AFTER TREATMENT	TEMPERATURE (° F.)		
	65°	65°–75°	75°
	CO_2 production 24 hours (dry-weight basis)		
1	118.7†	119.7†	139.6†
2	120.6†	121.8†	134.9†
3	160.3†	136.9†	130.3†
4	180.5†	....	139.7†
5	....	....	109.9
	CO_2 production 24 hours (single-plant basis)		
1	111.6†	114.1†	143.7†
2	102.8	103.0	109.2
3	127.9†	109.7†	108.5*
4	142.6†	....	88.7*
5	....	....	67.8†
	Dry weight		
1	94.6	95.3	103.2
2	84.6*	84.8*	81.0*
3	80.0†	80.2†	83.6†
4	79.1†	....	64.0†
5	....	....	61.8†

* Significant at 5% level.　　† Significant at 1% level.

The accumulation of solid materials in the basal region of the stems was influenced by spraying the plants with 2,4-dichlorophenoxyacetic acid. This effect was observed only in those plants

that had been treated with small amounts of the acid. In those sprayed with the highest concentration (1000 p.p.m.), the accumulation of solid matter in this region of the plant was depressed, indicating that the mobilization of food materials may have been affected by the application of relatively large amounts of the acid.

The water content of the leaf tissues was reduced by spraying the plants with the acid, while that of the stem tissues increased, in comparison with like parts of untreated plants. The significance of this response in relation to the ultimate toxic effects of the acid is not apparent.

The respiration of seedling bean and mature annual morning-glory plants was markedly accelerated when they were sprayed with 2,4-dichlorophenoxyacetic acid. In the case of beans, treated plants continued to respire at a greater rate, on the basis of amount of CO_2 per unit of dry weight, than did untreated ones, even to the point where they were beyond recovery. It should be emphasized that the bean seedlings were relatively low in carbohydrate reserves, since they were grown under relatively low light intensities in a greenhouse. Thus it is possible that the depletion of carbohydrate reserves previously observed in morning-glory (4) may be due largely to this respiratory response.

It is concluded that, of the responses studied, those which most seriously affected the plants include the effect of the acid in depressing the rate of leaf expansion and development and its interference with the usual pattern of transport and utilization of food materials.

Summary

1. Bean (*Phaseolus vulgaris*) seedlings were sprayed with 2,4-dichlorophenoxyacetic acid, mixed with Carbowax as a dispersing agent, to determine the toxic effects associated with some of the various physiological responses that the acid induces.

2. Within 1 hour, seedlings sprayed with a 1000 p.p.m. concentration showed marked epinastic responses and stem bending, which became more severe during the next 2 or 3 days. At the end of 5 days the plants were permanently wilted, and in 7 days they were dead.

3. The total amount of water absorbed and transpired by sprayed plants during the 5 days immediately following treatment was 34% less than that for comparable untreated ones. Water uptake was not immediately limited by treatment, since the seedlings continued to transpire and their leaves did not wilt until 5 days after spraying.

4. Leaf growth and expansion were markedly inhibited in both partially expanded leaves and those contained in terminal buds, even when sprayed with a concentration as low as 25 p.p.m.

5. The solid matter content of the above-ground part of the plant decreased markedly after spraying with a relatively high concentration (1000 p.p.m.), but increased accumulation of solid matter was noted in the basal region of the stems of those plants sprayed with lower concentrations (25, 50, and 250 p.p.m.).

6. The rate of accumulation of water in the leaves of sprayed plants was depressed, while in the stem tissues it was accelerated. On an over-all basis, however, the treated plants had higher percentages of moisture than the untreated ones.

7. The rate of respiration of seedling bean plants measured at three different temperatures was significantly increased as the result of spraying them with an aqueous mixture containing 0.1% (1000 p.p.m.) of 2,4-dichlorophenoxyacetic acid

and 0.6% Carbowax 1500. This increase in respiration was manifest 24 hours after treatment. The CO_2 output of treated plants was approximately 19–80% greater than that of untreated ones when sampled during 4 days.

8. Plants of annual wild morning-glory (*Ipomoea lacunosa*) sprayed in a similar manner with the acid at the time of

flowering showed an 80.6% increase in respiration during a 2-hour period on the fourth day after treatment.

Mr. JOHN N. YEATMAN assisted in preparing samples and recording the data of some of these experiments.

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FURTHER INVESTIGATION OF TOXIC SUBSTANCES WHICH ARISE FROM GUAYULE PLANTS: RELATION OF TOXIC SUBSTANCES TO THE GROWTH OF GUAYULE IN SOIL

JAMES BONNER

Introduction

It has been shown in a previous paper[1] that water or nutrient solution in contact with guayule roots accumulates substances which are toxic to the growth of guayule plants. These substances have been isolated in pure form, and one has been identified as trans-cinnamic acid, a normal constituent of the guayule plant. The application of pure crystalline cinnamic acid in nutrient solution to gravel cultures of guayule plants resulted in growth inhibition of such plants, as little as 1 mg. of the acid per liter resulting in

[1] BONNER, J., and GALSTON, A. W., Toxic substances from the culture media of guayule which may inhibit growth. BOT. GAZ. 106:185–198. 1945.

measurable growth depression. The present report describes work concerning the toxic substances, especially cinnamic acid, in relation to the growth of guayule in soil—and particularly in the field.

By use of the guayule seedling toxicity assay, described in detail in the earlier paper, it is possible to determine in a semi-quantitative manner the presence or absence of guayule growth inhibitors in preparations such as plant extracts. Guayule seedlings are grown in sand in flats until they reach a height of 15–20 mm., about 3 weeks after sowing. They are then transplanted singly to vials containing 15 cc. of four-times diluted Hoagland solution but in which the iron and

minor elements are maintained at full strength. The plants are held in place by a wad of cotton in a slit in the side of a cork which fits into the top of each vial. Twenty such vials are contained in a box of such dimensions that the top of the vial is just flush with the top of the box. In this way the roots are maintained in darkness. The plants are grown in an air-conditioned greenhouse at a constant temperature of 80° F. for 14 days. Growth is measured by the increase in

TABLE 1

TOXICITY ASSAY ON PURE CINNAMIC ACID, WITH AND WITHOUT ADDITION OF 2 GM. HANFORD SANDY LOAM PER ASSAY PLANT. EXPERIMENT G-149

CINNAMIC-ACID CONCENTRATION (MG./ASSAY PLANT)	No. ASSAY PLANTS	GROWTH PER 10 PLANTS (MM.)	
		No added soil	Two gm. soil per plant added
None (check)..	80	30	41
0.5............	20	23	26
1.0............	20	16	9
2.0............	20	9	4

height of each plant during the 14-day incubation period.

The assay plants, as the plants used in this test will be called, are grown in non-aerated solution, since previous experiments have shown that aeration does not promote growth of guayule seedlings under the present conditions. In each experiment, each substance was ordinarily tested on ten plants. The degree of reproducibility to be expected from means based on ten plants has been discussed in the previous paper. In general, a difference in growth in height of 50% or more between two such means may be taken as significant at the 1% level, while difference of 30% between two

groups of ten may be taken as significant at the 5% level.

The data summarized in this report are taken from experiments which were set up for statistical treatment by the analysis of variance. Such analyses have not been carried out, however, since the results are of a generally negative character.

Experimentation

TOXICITY OF CINNAMIC ACID IN PRESENCE OF SOIL.—In previous work it has been shown that cinnamic acid is highly toxic to guayule seedlings in the standard toxicity assay. The data of table 1 show that this toxicity is not decreased in the presence of soil (Hanford sandy loam). For this first experiment, 2 gm. of soil was added directly to each culture vessel containing 15 cc. of nutrient. Table 1 shows that as little as 0.5 mg. of cinnamic acid per plant gave significant growth reduction. Amounts as small as 0.2 mg. were found to give significant inhibition in other experiments. In addition, it is evident that the addition of 2 gm. of soil to the nutrient solution resulted, in the absence of cinnamic acid, in somewhat increased growth as compared with the control, a fact which was frequently but not always observed in other experiments with other soil samples. The result of this experiment therefore indicates that the toxicity assay may be used to test for cinnamic acid in the presence of soil. The following experiments are based on this premise.

In a second type of experiment, soil to which various concentrations of cinnamic acid had previously been added was used as addendum in the assay. Cinnamic acid (as a neutral solution of sodium cinnamate) was added to Hanford sandy loam at the rate of 0, 0.1, or 1.0 mg. of acid per gram of dry soil. The soil

samples were then dried and tested for their effects on the growth of the assay plants. The results of one experiment are given in figure 1, in which each point is based on the measurement of twenty plants. The soil which contained 0.1 mg. of added cinnamic acid per gram of soil showed no particular toxicity as compared with control soil when tested at the rate of 100 mg. or 300 mg. of soil per culture vessel. A marked toxicity was evident at the 3-gm. level, and a suggestion of toxicity was evident even at the level of 1 gm. per assay plant. When the soil contained 1 mg. of cinnamic acid per gram, toxicity was evident when as little as 100 mg. was added to each culture vessel. The data of figure 1 corroborate those of table 1 in indicating that amounts of cinnamic acid as little as 0.3 mg. or less may be measured in the assay, even if the cinnamic acid has been mixed in soil. The toxicity of soil containing as little as 0.01% cinnamic acid can then be detected.

EFFECT OF CINNAMIC ACID ON GROWTH OF PLANTS IN SOIL.—It may be shown that the addition of cinnamic acid to soil, either as an initial dose or as repeated smaller doses, results in growth inhibition of guayule seedlings planted in such soil. Such an experiment is reported in table 2. For this experiment a Hanford sandy loam was used. The soil was placed in pots, each holding approximately 1500 gm. To each of the twenty-one pots of one series was added 100 cc. of a solution containing 1 gm. of cinnamic acid (as potassium cinnamate) per 100 cc. All solution thus applied was retained by the soil, and the soil contents of each pot were therefore impregnated with 1 gm. of the acid. Similar series were prepared with 100 mg. and 10 mg. of acid per pot. One seedling guayule was transplanted to each pot and no further acid applied.

In further series, the acid was applied daily in the water with which the plants were irrigated. The entire experiment was set in a randomized block design in the greenhouse. The plants were supplied with a complete nutrient three or four times a week. Growth was estimated by measuring the height of the plants and by the dry weight after 8 weeks of growth. The results are given in table 2. It may be seen that 1 gm. of cinnamic acid per pot resulted in very considerable reduc-
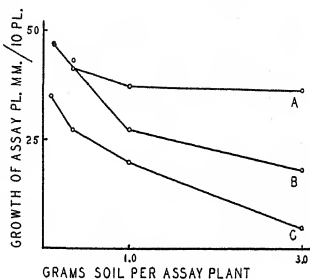

FIG. 1.—A, check soil, no added cinnamic acid; B, 0.1 mg. of cinnamic acid per gram of soil; C, 1.0 mg. of cinnamic acid per gram of soil.

tion in growth, both in height and in dry weight. Significant, although small, growth reductions were also effected by 100 mg. and by 10 mg. Daily application of water containing as little as 10 mg. of acid per liter (as potassium cinnamate) also resulted in significant growth reductions. These results, which were confirmed in a second experiment, show that growth inhibition of guayule by cinnamic acid may be achieved in soil as well as in a gravel substratum, as has been reported in the earlier paper.

TOXICITY ASSAYS ON SOIL WHICH HAS SUPPORTED GROWTH OF GUAYULE.—The soils of the experiment of table 2, to which cinnamic acid had been added and

in which this addition had resulted in inhibition of growth of guayule, were assayed for toxicity. To this end, when the plants were harvested, soil samples were removed from each pot and the twenty-one individual samples of each treatment composited into three lots. Each sample

to the soil in this experiment were sufficiently great so that they should have been readily detectable. Thus, approximately 1.3 mg. of cinnamic acid had originally been added to the 2 gm. of soil placed in each vial in the toxicity assay (table 3). This amount should have been

TABLE 2

GROWTH OF GUAYULE SEEDLINGS IN HANFORD SANDY LOAM TO WHICH CINNAMIC ACID HAD PREVIOUSLY BEEN ADDED, OR TO WHICH IT WAS ADDED IN THE WATER. PLANTS GROWN SINGLY IN CANS CONTAINING APPROXIMATELY 1500 GM. DRY SOIL. PLANTED DEC. 1; 21 PLANTS PER TREATMENT. EXPERIMENT G-155 (THREE PLOTS)

Added to soil	Added to water	Height after 6 weeks (cm./pl.)	Dry wt. per plant after 8 weeks (gm.)
None	None	18.2±0.473	0.73±0.0746
1 gm./pot	None	6.3±1.400*	0.33± .0907*
100 mg./pot	None	15.1±0.846*	0.60± .0765
10 mg./pot	None	14.1±1.492†	0.60± .0971
None	100 mg./l.	13.3±1.373*	0.46± .0833†
None	10 mg./l.	14.7±1.214†	0.56± .0895
None	1 mg./l.	15.1±1.325†	0.64±0.1096

* Difference between this value and corresponding one for control plants significant at 1% level; † at 5% level.

TABLE 3

TOXICITY ASSAY (2-GM. SAMPLES) ON HANFORD SANDY LOAM TO WHICH CINNAMIC ACID HAD BEEN ADDED AND IN WHICH GUAYULE PLANTS HAD PREVIOUSLY BEEN GROWN. EXPERIMENT G-148, TESTING SOIL OF EXPERIMENT G-155

Cinnamic acid added per 1500 gm. of soil in exp. G-155	Plants grown in this soil in previous exp. Wt. after 8 weeks (gm./pl.)	No. samples	No. assay plants	Growth (mm./10 pl.)
1. None (check), no soil			90	34
2. Check soil, no acid	0.73	3	30	28
3. 1 gm. acid/pot	0.33	3	30	32
4. 0.1 gm. acid/pot	0.60	3	30	27
5. Soil watered with solution of 100 mg./l.	0.46	3	30	28
6. Soil watered with solution of 10 mg./l.	0.56	3	30	31
7. Soil watered with solution of 1 mg./l.	0.64	3	30	34

was tested for toxicity in the assay by the addition of 2 gm. of soil to each vial. Nutrient solution was added and seedling plants grown in them for 2 weeks in the standard manner. Table 3 shows that none of the treated soils showed appreciable toxicity. The amounts of acid added

detected by growth reduction (fig. 1). The fact that no toxicity was detected may indicate that cinnamic acid is destroyed by soil, a thesis which is supported by the experiment in the next section.

DESTRUCTION OF CINNAMIC ACID BY

SOIL.—Cinnamic acid, in the form of sodium cinnamate, was incorporated in Hanford sandy loam to the extent of o, 0.1, or 1.0 mg. per gram dry weight of soil. Water was then added to bring each sample to field capacity. An aliquot of soil from each treatment was immediately dried in an oven at 70° C. Other aliquots were permitted to incubate for 2, 4, 7, or 14 days at 25° C. before being subjected to drying. Each sample was then tested for toxicity in the standard assay. The results (table 4) show that the

crobial activity, in Hanford sandy loam. This conclusion indicates that accumulation of cinnamic acid or related toxic substances in soil under field conditions of guayule culture would necessarily be small, at least in soils possessing a suitable microflora for cinnamic-acid destruction.

TOXICITY ASSAYS ON GUAYULE SOILS UNDER FIELD CONDITIONS.—Soil samples (Hanford sandy loam) from five guayule plantings, in different geographical areas and in three different soil types, were

TABLE 4

DISAPPEARANCE OF TOXICITY DURING INCUBATION OF SOIL (HANFORD SANDY LOAM) TREATED WITH CINNAMIC ACID AND INCUBATED AT FIELD CAPACITY AND ROOM TEMPERATURE AFTER ADDITION OF CINNAMIC ACID. EXPERIMENTS 151, 152. EACH FIGURE BASED ON MEASUREMENTS ON 20 PLANTS. CONTROL PLANTS (NO SOIL ADDED) GREW 37 MM./10 PLANTS (130 PLANTS)

CONCENTRATION OF CINNAMIC ACID ADDED TO SOIL	GROWTH PER 10 PLANTS (MM.)				
	Soil incubated				
	0 days	2 days	4 days	7 days	14 days
None................	32	32	32	32	32
0.1 mg./gm............	22.5	31.5	26.5	28.5	37
1.0 mg./gm............	8	9.5	17.5	19.5	35.5

toxicity of the added cinnamic acid disappeared during incubation of the soil. When 0.1 mg. of the toxic agent was added per gram of soil, the toxicity largely disappeared within 2 days. The toxicity of soil containing 1 mg. of cinnamic acid per gram disappeared during 2 weeks of incubation.

In a separate experiment it was also shown that in soil treated with cinnamic acid, brought to field capacity and then sterilized by autoclaving, no reduction of toxicity due to added acid occurred in the course of incubation. Added cinnamic acid was similarly stable in oven-dry soil. These facts indicate that cinnamic acid is rapidly destroyed, probably by mi-

tested in the toxicity assay. The first experiment was carried out with soils from a field planting approximately $2\frac{1}{2}$ years old in Arcadia, California. Soil samples were removed from the 6- and the 12-inch levels directly under guayule plants and from between plants in the rows. Samples of check soil, never planted to guayule, were removed from the edges of the planting. All samples were tested in the standard assay, and the results are given in table 5. No consistent differences between the effects of check soil and soil from the neighborhood of guayule plants could be found. It must be concluded that in this planting cinnamic acid or related toxic substances did not accumu-

late in concentrations equivalent to the
0.1 mg. of cinnamic acid per gram of soil
(0.01%) necessary for detection. Since
amounts of cinnamic acid as low as
0.001% or lower were found to be effec-
tive in retarding slightly the growth of
soil-grown guayule plants (table 2), it is
possible that amounts of toxic materials
great enough to exert a small influence on
the growth of the soil-grown plants
might have been contained in the soil
without being detectable.

TABLE 5

TOXICITY ASSAYS OF HANFORD SANDY LOAM (2
GM./PL.) FROM GUAYULE TEST PLANTING. AR-
CADIA PLOT, SAMPLED DEC., 1944; PLANTED
JUNE, 1942. EXPERIMENTS G-144 AND 145

Soil derivation	Level (inches)	No. assay plants	Growth per 10 plants (mm.)
None (check), no soil..	....	90	32
Check soil, never in guayule (sample a).	6	20	36
	12	20	36
Check soil, never in guayule (sample b).	6	20	39
	12	20	27
Under field plant a...	6	20	36
	12	20	34
Under field plant b...	6	20	46
	12	20	45
Between plants in row	6	20	31
	12	20	46

A second series of samples were col-
lected from six locations in the Whittier
nursery in Indio, California, in January,
1945. This nursery is in a soil of the Indio
series. At each location, samples were re-
moved from under the center of rows in
the nursery beds, from between rows,
and from adjoining plots never in guay-
ule. Samples were taken at both the 6-
and 12-inch level throughout. Table 6
shows the results of toxicity assays on
these samples. No detectable reduction
in growth of the seedlings was achieved
by additions of any of the nursery soils.

Tests for possible toxicity toward
guayule seedlings were conducted on fur-
ther series of soil samples collected in the
Salinas area. All the samples consisted
of soils of the Chualar loam series. The
first set was taken from standard beds in
the Alisal nursery. A second set was tak-
en from an experimental portion of the
Alisal nursery in which seed had been
broadcast rather than drilled, and which
contained a dense stand of 2-year-old
plants. A third set of samples was taken
from an 8-year-old planting in Spence
field. All the samples were tested in the
toxicity assay, and the results are given
in tables 7 and 8. It may be seen that no
appreciable toxicity was found in any of
the series.

TOXICITY OF SOIL IN POT CULTURES.—
During the last 5 years, numerous experi-
ments have been carried out with guay-
ule grown in soil contained in crocks or
cans of various sizes. During the present
investigation such soil was tested for
toxicity in the standard assay. The soil
was Hanford sandy loam contained in
1-gallon cans. Guayule plants had grown
in the soil, one plant per can, for rather
more than 2 years. Soil from ten cultures
was sampled and tested separately. Ta-
ble 9 shows that in two cases the soil ex-
hibited marked toxicity. In six other cul-
tures no toxicity was found and two cul-
tures showed questionable toxicity. No
further investigation of the reason for
this variability in accumulation of tox-
icity in pot cultures has as yet been at-
tempted. It would seem, however, that
occasionally toxicity may accumulate in
soil under pot-culture conditions where
the permeation of soil by roots is more
thorough than under field conditions.
Soil from cultures similar to those of ta-
ble 9, but in which guayule had grown
for only 6 months and in which explora-
tion of the soil by the roots was less thor-
ough, in no case showed toxicity.

INFLUENCE OF GUAYULE ROOTS ON GROWTH OF GUAYULE SEEDLINGS.—In the earlier paper it has been shown that water in which guayule roots have been soaked contains substances, including cinnamic acid, which are toxic to guayule plants. In the experiments of table 10, fresh guayule roots were added directly to the culture solution of vials in which guayule assay plants were grown. Addi-

TABLE 6

TOXICITY ASSAY ON SOILS FROM WHITTIER NURSERY. SOIL SAMPLES TAKEN JAN., 1945, FROM BED IN WHICH PLANTS HAD BEEN GROWING APPROXIMATELY 2 YEARS. EXPERIMENTS G-146, 147. GROWTH OF CONTROL PLANTS (NO SOIL ADDED) WAS 47.6 MM. PER 10 PLANTS

| PLOT IN NURSERY | GROWTH OF ASSAY PLANTS (MM./10 PLANTS) | | | | | |
| | Control soil (never in guayule) | | Center of guayule bed | | Between rows in guayule bed | |
	6″ level	12″ level	6″ level	12″ level	6″ level	12″ level
1	66	58	70	42	44	39
2	51	45	49	45	54	57
3	68	52	59	68	47	51
4	50	37	41	44	42	46
5	59	71	42	43	49	40
6	48	72	50	45	55	64
Average..	57.0	55.8	51.8	47.8	48.5	49.5

TABLE 7

TOXICITY ASSAY ON SOILS OF CHUALAR LOAM SERIES FROM VARIOUS GUAYULE PLANTINGS IN SALINAS AREA. SOIL SAMPLES TAKEN MAY, 1945. EXPERIMENT G-154

PLOT	GROWTH OF ASSAY PLANTS (MM./10 PLANTS)					
	Soils from 6-inch level					
	Part A; Alisal nursery beds		Part G; Alisal nursery beds (broadcast seeds; heavy stand of plants)		Part K; Spence field (8-year-old planting)	
	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed	Check (never in guayule)	From under plants
1	37	30	30	28	33	31
2	38	23	30	30	40	28
3	33	31	22	33	27	37
4	26	28	32	22	29	24
5	28	37	28	29	24	22
6	32	26	22	31	33	24
Average..	32.3	29.2	27.3	28.8	31.0	27.7

tion of as little as 100 mg. of fresh root per culture vessel reduced growth by about two-thirds, while addition of 1 gm. per vial resulted in death of all the plants. It might be expected, therefore, that guayule-root residues in soil should leave behind residual toxicity toward future plantings. Such toxicity has not been found in two experiments with Hanford sandy loam. In neither case was it pos-

TABLE 8

TOXICITY ASSAY ON SOILS OF CHUALAR LOAM SERIES FROM VARIOUS GUAYULE PLANTINGS IN SALINAS AREA. SOIL SAMPLES TAKEN MAY, 1945. EXPERIMENT G-153

	GROWTH OF ASSAY PLANTS (MM./10 PLANTS)					
	Soils from 12-inch level					
PLOT	Part A; Alisal nursery beds		Part G; Alisal nursery beds (broadcast seeds; heavy stand of plants)		Part K; Spence field (8-year-old planting)	
	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed
1..........	19	19	23	19	24	20
2..........	28	20	27	18	19	24
3..........	20	25	25	26	36	29
4..........	18	19	28	12	25	30
5..........	19	18	25	28	40	19
6..........	24	19	29	28	42	31
Average..	21.3	20.0	26.2	21.8	31.0	25.5

TABLE 9

TOXICITY ANALYSIS OF HANFORD SANDY LOAM IN WHICH GUAYULE PLANTS HAD PREVIOUSLY GROWN IN 2-GALLON CANS FOR APPROXIMATELY 2 YEARS. TWO GM. SOIL ADDED PER TEST PLANT

Experiment no.	Soil from can no.	No. tests	Growth of assay plants in % of controls (soil not added)
G-142, 143....	1	2	14
G-144..........	2	1	117
G-145..........	3	1	109
G-149..........	4	1	100
G-149..........	5	1	116
G-149..........	6	1	126
G-149..........	7	1	103
G-157, 158....	8	2	77
G-157, 158....	9	2	51
G-157, 158....	10	2	88

sible to demonstrate any unfavorable effect over a period of 3–6 months. This may again reflect destruction of the toxic principles by soil.

Discussion

In the earlier paper evidence was presented which indicated that, in gravel cultures of guayule, organic compounds accumulate which are toxic or inhibitory to the growth of the same or other guayule plants. Fresh guayule roots, or water extracts of fresh roots, are also highly toxic to guayule plants in sand or nutrient solution culture. It has not been possible in the present work to demonstrate any accumulation of such toxic compounds in soil in which guayule plants

had been grown under nursery or field conditions. The method of testing for the toxic compounds has been shown to be sensitive to as little as 0.01% of one of the toxic agents, cinnamic acid, based on weight of dry soil. Even lower concentrations should be indicated qualitatively by the assay. It may be said with certainty, therefore, that of the soils tested from plants in Arcadia, Indio, and Salinas, none contained as much as the toxicity equivalent of 0.01% cinnamic acid, even though the plants had grown in these soils for periods of 2–8 years. On the other hand, Hanford sandy loam and soil capable of supporting good growth of guayule was shown to destroy rapidly the toxicity of added cinnamic acid, possibly by microbial activity. It would seem obvious that the toxic materials released from the guayule plant must be destroyed or inactivated in the soil under favorable field conditions. It is conceivable that infertility of a particular soil toward guayule might be related to accumulation of cinnamic acid or other toxic substances owing to the absence or failure of the system for neutralizing or destroying the toxic substances. This possibility has not been investigated; all the soils used in the present investigation were of high potential fertility with regard to guayule.

TABLE 10

TOXICITY ASSAY ON FRESH ROOTS, EXPERIMENT G-152

Source of roots added to assay plants	Amount of roots added per assay plant (mg. fresh roots)	No. assay plants	Growth of assay plants in % of controls (soil not added)
Seedlings in flat	100	10	30
	300	10	16
	1000	10	0
One-year field plants	100	10	33
	300	10	21
	1000	10	0

Summary

Guayule plants have previously been shown to produce inhibitory substances in gravel culture. In the present experiments it has not been possible to detect these inhibitory substances in any of three soils in which guayule had grown in the nursery or field for periods of 2–8 years.

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EFFECT OF TREATING SOIL AND SEEDS WITH 2,4-DICHLORO-PHENOXYACETIC ACID ON GERMINATION AND DEVELOPMENT OF SEEDLINGS[1]

C. L. HAMNER, J. E. MOULTON, AND H. B. TUKEY

A previous paper (1) reported that germinating seedlings of white sweet clover (*Melilotus alba*) emerging 3–7 days after the soil surface had been sprayed with 2,4-dichlorophenoxyacetic acid were completely killed. This suggests the possibility of using this chemical to destroy weeds by soil treatment. The experiments reported here investigated these possibilities.

Investigation

APPLICATION TO UPLAND SOIL

PROCEDURE.—Initial trials were conducted under greenhouse conditions at Geneva, New York, between December, 1944, and February, 1945, and consisted of various soil treatments with 2,4-dichlorophenoxyacetic acid. The soil was a potting mixture of one-quarter sand, one-quarter peat moss, and one-half heavy clay loam. The acid was thoroughly incorporated in the soil, either in a talc-dust mixture or in a water solution with Carbowax 1500. Each soil treatment consisted of ten pots replicated seven times. The concentrations of the acid were 0.1, 1, 10, 100, and 1000 mg. per 4-inch pot of soil.

The water solution in Carbowax was prepared by adding 5 gm. of Carbowax 1500 containing the desired amount of 2,4-dichlorophenoxyacetic acid to 1 liter of water. The talc dust was prepared by adding 5 gm. of Carbowax 1500 containing the desired amount of acid to 100 gm. of the talc. Enough alcohol was

added to the dry mixture to wet it. It was then thoroughly mixed and placed in an oven at 80° C. and dried, the alcohol evaporating and leaving the dust mixture.

Seeds of white sweet clover (*Melilotus alba*), Certified Yorktown wheat (*Triticum vulgare*), and Danish Ballhead cabbage (*Brassica oleracea*) were used. On December 26, 1944, twelve seeds of clover, ten of wheat, and ten of cabbage were planted in each pot.

RESULTS.—The solution had greater inhibiting effect than the dust, perhaps as a result of greater distribution of the chemical.

When the acid was applied in solution to the soil, no germination of wheat, cabbage, or clover occurred at a concentration of 1 gm. (or 0.1 gm. of the chemical per pot of soil). At a concentration of 0.01 gm. of the acid in solution, cabbage and clover seeds failed to germinate; however, 85% of the wheat germinated, although growth was inhibited somewhat (fig. 1). On the other hand, when the acid was applied as a dust, complete inhibition of germination of all three species occurred only in the pots containing as much as 1 gm. of the acid. Eighty-five per cent of the wheat germinated in pots containing 0.01 gm. of the chemical carried in the dust, while only 18% of the cabbage and 10% of the clover germinated. At 0.001 gm. of the acid per pot, no effect on germination was observed in any of the species (table 1; figs. 2, 3).

APPLICATION TO MUCK SOIL

PROCEDURE.—Muck soil was also treated with 2,4-dichlorophenoxyacetic acid. On October 15, 1945, soil infested with seed was collected in the vicinity of East Lansing, Michigan. Twenty-five pounds of muck were used in each treatment. To each lot of muck, acid in a 0.5% Carbowax 1500 solution was added at the rate of 1, 10, and 100 mg. of 2,4-dichlorophenoxyacetic acid per 1000 gm. muck containing 36% water. One untreated lot was left as a check. The muck was then placed in flats in the greenhouse and kept moist. The air temperature varied between 60° and 80° F., with a mean of about 72° F. The flats were examined at the end of 4 weeks and the kinds and relative number of weeds recorded for each.

To test the residual effect of the acid in the muck, bean and pea seeds were planted in the flats 1, 2, 3, and 4 weeks after the treatment.

RESULTS.—After 4 weeks many plants, including lambs quarters (*Chenopodium album*), sow-thistle (*Sonchus arvensis*), tomato (*Lycopersicum esculentum*), purslane (*Portulaca oleracea*), foxtail (*Setaria lutescens*), and red root (*Amaranthus retroflexus*) were growing in the control flats. Where 1 p.p.m. of 2,4-dichlorophenoxyacetic acid was applied, there were only about 20% as many plants as in the checks; the flats containing 10 and 100 p.p.m. of the chemical were entirely free from weed growth (fig. 6).

Peas and beans planted in muck 1 week after it had been treated with the acid at 100 p.p.m. were severely affected; the peas failed to germinate and the beans exhibited pronounced formative defects (fig. 8).

The toxic effect of the acid in the muck disappeared to a great extent after 3

weeks. Bean seeds planted in muck 3 weeks after it had been treated with 100 p.p.m. of 2,4-dichlorophenoxyacetic acid germinated and grew with only slight symptoms of the presence of the acid. Primary leaves of a few plants developed virus-like symptoms, indicating that
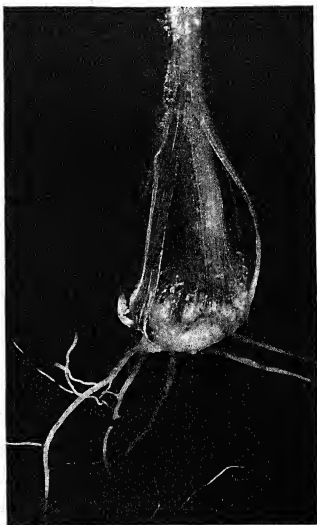

FIG. 1.—Swelling at base of wheat plant growing in soil containing 0.1 mg. 2,4-dichlorophenoxyacetic acid per pot of soil.

some of the chemical was still active in the muck.

Bean and pea seeds planted in the muck 4 weeks after the time of treatment germinated and grew normally (figs. 7, 9), both in the 10 and 100 p.p.m. flats, indicating that the toxic effect of the acid had been dissipated.

APPLICATION TO SEEDS

PROCEDURE.—On October 19, 1945, seeds of various grasses and crop plants were soaked in solutions containing 1, 10, and 100 p.p.m. of the acid for a period of 4 hours. The acid was dissolved in 5 gm. of Carbowax 1500 and this mixture added to 1 liter of water. The seeds were wrapped in cheesecloth and placed in the solutions. At least 200 seeds were used in each treatment and planted in moist sand for the germination test.
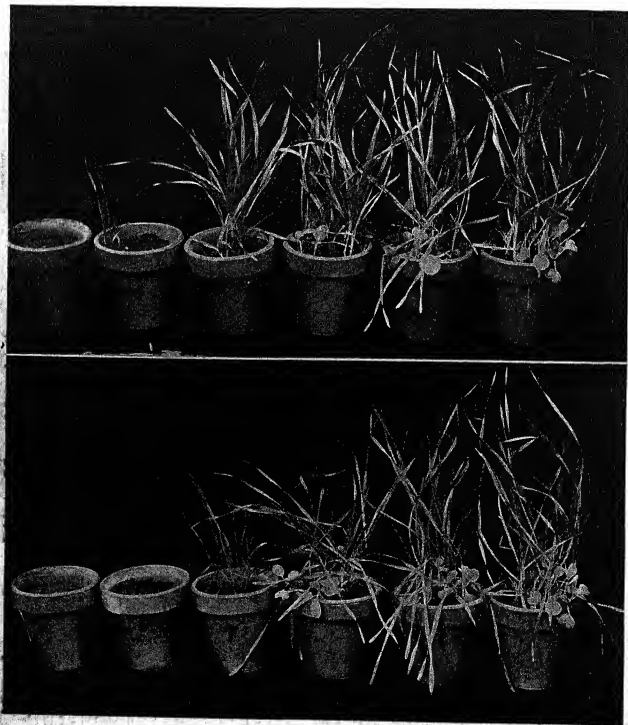
FIGS. 2, 3.—Fig. 2 (top), growth of wheat, cabbage, and clover in series of pots in which soil was treated with 2,4-dichlorophenoxyacetic acid, using talc as carrier. Concentrations of acid per pot (left to right): 1, 0.1, 0.01, 0.001, 0.0001 gm., and control. Fig. 3 (bottom), growth of wheat, cabbage, and clover in soil contained in 4-inch pots treated with the acid, using water as carrier. Concentrations of acid per pot (left to right): 1, 0.1, 0.01, 0.001, 0.0001 gm., and control.

RESULTS.—One week after treatment, examination of the plant material showed (table 2) that the grass seeds were as a whole more resistant to the acid than were the non-grass seeds. It should be emphasized, however, that grass seeds can be affected and in many cases completely killed. Sudan grass, for example, germinated when soaked in a solution of the acid at 100 p.p.m. for 4 hours, but germination was abnormal and top growth was inhibited. Moreover, no roots were produced.

The seeds of some dicotyledons, such as red kidney bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*), showed marked response. Bean seeds soaked in a 1 p.p.m. solution were affected. The seedlings exhibited the characteristic formative effects and "virus"-like symptoms (2). Seedlings of both bean and pea were severely checked by seed treatment at 10 p.p.m. The roots as well as the tops were checked. Swelling occurred in the bean hypocotyl. This was especially noticeable at the cotyledonary node. When the bean and pea seeds were soaked in a solution of the acid at 100 p.p.m., growth of the seedlings was almost completely checked. No top or root growth occurred in the pea, while only 1% of the beans made feeble growth. (fig. 10). Figure 4 illustrates a typical condition in the bean following treatment of the seeds at 100 p.p.m.

APPLICATION TO MANURE

Manure contains many weed seeds, its use often being objectionable for this reason (3).

In order to be certain that seeds would be present in the manure used in these trials, seeds of rape (*Brassica napus*), rye (*Secale cereale*), field pea (*Pisum sativum*), brome grass (*Bromus arvensis*), meadow fescue (*Festuca elatior*), creep-

ing bent grass (*Agrostis canina*), orchard grass (*Dactylis glomerata*), hairy vetch (*Vicia villosa*), and alsike clover (*Trifolium hybridum*) were added to the manure in large numbers. The seed-infested manure was then divided into three lots and one lot was treated with 2,4-dichlorophenoxyacetic acid at 10 p.p.m., an-


FIG. 4.—Bean seedling germinated in sand containing 100 p.p.m. of 2,4-dichlorophenoxyacetic acid showing proliferation of tissues in young root and hypocotyl.

other at 100 p.p.m., and a third left untreated. The manure was then mixed with sand, the final mixture being about one part manure to one part sand. This was placed in metal flats and kept moist in a warm greenhouse with a mean temperature of 72° F.

After 3 days, seeds in the control lot began to germinate, and after 2 weeks the entire surface was covered with plant growth. The seeds in the treated flats at
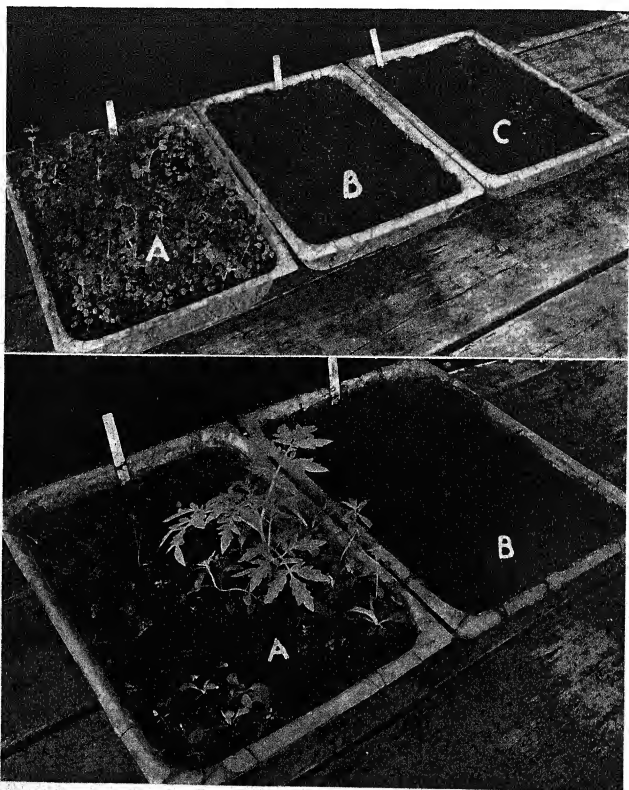
FIGS. 5, 6.—Fig. 5 (top), control of weeds by treatment of manure with 2,4-dichlorophenoxyacetic acid. Flats contained equal mixture of sand and manure. Each flat sown with equal amounts of several varieties of seeds. *A*, untreated. *B*, manure treated with 10 p.p.m. acid at time of seeding. *C*, manure treated with 100 p.p.m. at time of seeding. Fig. 6 (bottom), control of weeds in muck by treatment of soil with the acid. *A*, untreated. *B*, muck soil treated with 100 p.p.m.
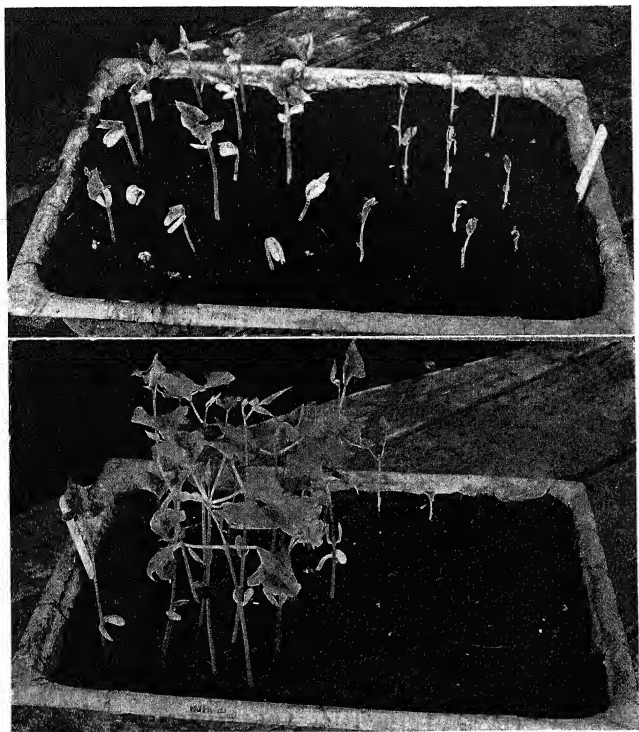
FIGS. 7, 8.—Fig. 7 (top), normal germination and growth of pea and bean in treated muck, planted 4 weeks after application of 100 p.p.m. of 2,4-dichlorophenoxyacetic acid to soil. Fig. 8 (bottom), residual effect of acid shown by abnormal growth of bean and pea planted 1 week after treatment of soil. Left, bean. Right, pea, showing almost complete inhibition of growth.
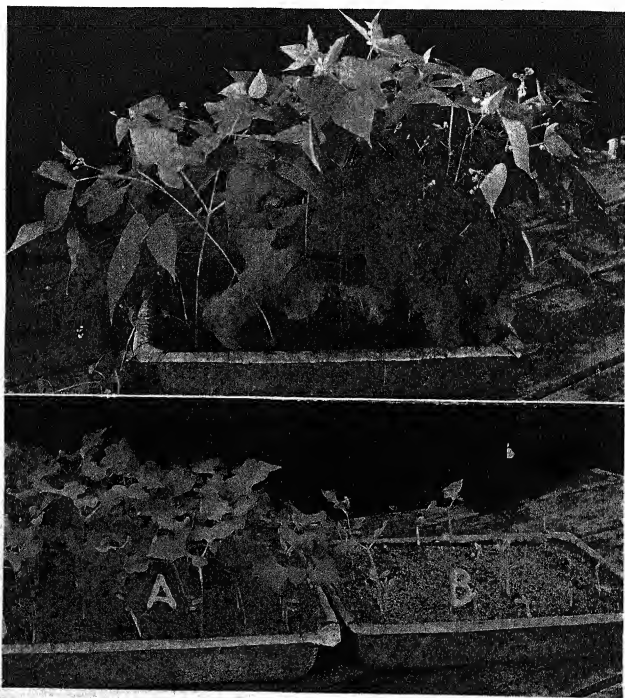
Figs. 9, 10.—Fig. 9 (top), absence of residual effect of 2,4-dichlorophenoxyacetic acid shown by normal growth of beans planted 4 weeks after treatment of soil. Fig. 10 (bottom), effect of seed treatment with acid on growth of bean seedlings. *A*, checks showing normal growth. *B*, inhibition of bean growth after soaking 4 hours in 100 p.p.m.

10 and 100 p.p.m. were inhibited in germination. Only a few of the grasses germinated and made feeble growth in the 10 p.p.m. flats (fig. 5).

Discussion

The normal germination of many seeds can be affected by soaking in low concentrations of 2,4-dichlorophenoxyacetic acid. Pronounced morphological changes occur when certain seeds are treated in solutions containing as low as 1 p.p.m. This is in sharp contrast to the 1000 p.p.m. used in the herbicidal sprays of mature plants. It is thus evident that certain germinating seeds and very young seedlings are much more responsive to the chemical than are more advanced seedlings and established plants. The herbicidal spray at a concentration of 1000 p.p.m. to the tops of plants is selective in action, being effective on many dicotyledonous plants while most grasses are apparently not responsive. Many grass seeds, on the other hand, are markedly affected at the comparatively low concentration of 100 p.p.m.

In our seed-treatment experiments a 4-hour soaking period was used and the seeds were then removed from solution and received no more of the chemical. On the other hand, when the chemical was applied to the soil, the seeds were in continuous contact with the acid solution and hence, for a given concentration, much greater inhibition of growth of the seeds resulted.

This suggested the application of a lethal dose of the acid to the soil, thus providing a method of eliminating weeds. Seed-infested muck soil treated in the greenhouse at 10 and 100 p.p.m. of the acid remained completely free of any weed growth. The residual effect of the chemical was tested and the acid found to be active in the muck for only a few

weeks, apparently either leaching out or changing in form. Thus is available a method of sterilizing the soil of weed seeds and leaving the soil unusable for only a relatively short period of time, the length of this period probably depending upon soil temperature and moisture conditions. This method could be used

TABLE 1

EFFECT OF SOIL TREATMENT WITH 2,4-DICHLO-
ROPHENOXYACETIC ACID IN CARBOWAX 1500
ON GERMINATION OF WHEAT, CABBAGE, AND
CLOVER

AMOUNT OF ACID PER POT (MG.)	PERCENTAGE GERMINATION		
	Wheat	Cabbage	Clover
	Aqueous solution		
0.1..............	90	70	84
1.................	87	72	85
10................	85	12	6
100...............	0	0	0
1000..............	0	0	0
Control............	86	72	85
	Talc-dust mixture		
0.1..............	86	73	83
1.................	90	71	80
10................	85	15	10
100...............	6	0	0
1000..............	0	0	0
Control............	88	70	83

where muck soil is used in top-dressing lawns and golf courses. Seed beds might be treated with 2,4-dichlorophenoxyacetic acid several weeks before they are to be planted, thus reducing the cost of weed control. Manure usually containing large numbers of weed seeds might likewise be treated with the chemical some time before it is spread. It is possible that large-scale application of the acid to muck and upland soil will be made, especially in

TABLE 2

GERMINATION AND GROWTH OF SEEDS SOAKED 4 HOURS IN 2,4-DICHLOROPHENOXYACETIC ACID

SEEDS	CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID PER LITER OF WATER (MG.)			
	0	1	10	100
Grasses:				
Sudan grass (Sorghum vulgare sudanense)				
Percentage germination	80	80	75	60
Root growth	++++*	++++	++++	++++
Top growth	++++	++++	+++	+
Fescue (Festuca elatior)				
Percentage germination	95	95	95	95
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	+++
Brome grass (Bromus arvensis)				
Percentage germination	80	80	80	60
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Timothy (Phleum pratense)				
Percentage germination	90	90	90	90
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Rye (Secale cereale)				
Percentage germination	90	90	90	75
Top growth	++++	++++	++++	+++
Root growth	++++	++++	++++	+++
Wheat (Triticum vulgare)				
Percentage germination	90	90	90	80
Top growth	++++	++++	++++	+++
Root growth	++++	++++	++++	+++
Other seeds:				
Red kidney bean (Phaseolus vulgaris)				
Percentage germination	90	85	60	7
Top growth	++++	+++	++	+
Root growth	++++	+++	++	+
White bean (Phaseolus vulgaris)				
Percentage germination	92	92	65	1
Top growth	++++	+++	++	+
Root growth	++++	+++	++	+
Tomato (Lycopersicum esculentum)				
Percentage germination	67	60	62	50
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Cabbage (Brassica oleracea var. capitata)				
Percentage germination	40	43	22	0
Top growth	++++	+++	++	....
Root growth	++++	+++	++	....
Sprouting broccoli (Brassica oleracea var. italica)				
Percentage germination	90	90	60	30
Top growth	++++	++++	+++	++
Root growth	++++	++++	+++	++

TABLE 2—*Continued*

SEEDS	CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID PER LITER OF WATER (MG.)			
	0	1	10	100
Pea (Pisum sativum)				
Percentage germination...........	91	82	8	2
Top growth.....................	++++	+++	++	+
Root growth....................	++++	+++	++	+
Sweet clover (Melilotus alba)				
Percentage germination...........	90	80	5	0
Top growth.....................	++++	+++	+	....
Root growth....................	++++	+++	+	....
Rape (Brassica napus)				
Percentage germination...........	90	90	90	70
Top growth.....................	++++	++++	++++	++
Root growth....................	++++	++++	++++	++
Vetch (Vicia villosa)				
Percentage germination...........	90	90	80	20
Top growth.....................	++++	++++	+++	++
Root growth....................	++++	++++	+++	++
Alsike clover (Trifolum hybridum)				
Percentage germination...........	90	90	90	80
Top growth.....................	++++	++++	++++	+++
Root growth....................	++++	++++	++++	+++

* ++++, no inhibition; +++, slight inhibition; ++, moderate inhibition; and +, strong inhibition.

land used for crops requiring considerable hand weeding.

Summary

1. Many seeds are affected when planted in mineral or muck soil previously treated with 2,4-dichlorophenoxyacetic acid. Concentrations of the acid as low as 1 p.p.m. in the soil affect the germination and growth of many seeds.

2. Although grass seeds can be destroyed, they are more resistant to the acid than are many other seeds.

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SEASONAL VARIATION IN THE ENZYME CONTENT OF ELEVEN VARIETIES OF CARROTS

HERMAN J. MORRIS, C. A. WEAST, AND HANS LINEWEAVER

Introduction

The inequalities of enzyme concentration in tissues of different species of plants, as well as in the same species at different stages of maturity, are well recognized. For example, BACH *et al.* (1, 2) observed considerable changes in amylase and protease activity of wheat during ripening and germination; in contrast, only slight changes occurred in the catalase and peroxidase content. IWANOFF (14) observed a twenty fold decrease in catalase and saccharase of fruit of pumpkin during ripening. BAILEY and MCHARGUE (3) found a steady decrease of catalase and peroxidase, and a steady increase of invertase, during the ripening of tomato fruits. OVERHOLSER (20) reported a marked decrease in catalase with maturity of eight varieties of pears. These variations in enzyme activity with maturity are generally greater than differences between varieties. Thus, SALLANS and ANDERSON (23) found a maximum difference of only about twofold in the saccharifying activities of twelve varieties of barley grown under the same conditions.

Reported increases in enzyme activity do not always mean a parallel increase in enzyme content, of course. For example, in the germination of certain grains, a-amylase not only increases but becomes more water-soluble. In measuring enzyme content, re-extractions, extractions under various conditions, and determinations of enzyme activity of finely divided suspensions of tissue will give some clues to completeness of extraction. Even though determinations on suspensions

show that extraction is complete, adsorbed inactive enzyme may still remain in the tissue. Thus, WILLSTÄTTER and WALDSCHMIDT-LEITZ (25) found that lipase was partially active while adsorbed on Kaolin or alumina but was quite inactive when adsorbed on fat or cholesterol. On the other hand, many enzymes are active or partially so when still attached to the cell particles (for example, pectinesterase, peroxidase, ascorbic-acid oxidase). Care must be taken, of course, that inactivation does not occur during extraction. In the last analysis, what is meant by the total enzyme content of vegetable tissue is largely defined by the methods used to prepare the extracts. Considerable attention must therefore be given to the methods used to obtain enzyme-content data.

The study reported here is limited to a comparison of enzyme contents, as related to variety and growing conditions (temperature and photoperiod), of the peeled portion of relatively young carrots. Eleven varieties were grown and harvested under identical conditions and their contents of the enzymes, peroxidase, catalase, ascorbic-acid oxidase, and phosphatase determined. Lipoxidase was not detected, and only a trace of catecholase was present in the peeled portion. The variations in enzyme-content values are discussed in relation to the environmental conditions and metabolism of the carrots and, in the case of ascorbic-acid oxidase, to their ascorbic-acid content. This work was undertaken as part of a study to determine what kind of carrots would be most suitable for de-

hydration. However, in spite of variations in enzyme activity, all varieties studied appeared to be equally suitable for dehydration.

Experimental procedure

The eleven varieties of carrots were grown under controlled conditions on the same plot of ground at two different seasons of the year at Milpitas, California, by the Associated Seed Growers, Inc. The first planting was on August 21, 1942, and the second on March 23, 1943. Carrots from each planting were harvested at two stages of maturity: January 18 and March 25 for the first planting and July 26 and September 20 for the second. The product in each case was transported to the laboratory and held at 1° C. for 3–6 days. The carrots were then washed, abrasively peeled, hand-trimmed, diced ($\frac{3}{8}$ inch), mixed, and sampled for the enzyme and ascorbic-acid analyses. Ascorbic acid was determined by a modification (19) of the 2,6-dichlorophenol indophenol method, and the enzyme contents were determined as follows:

Extracts were prepared by comminuting duplicate 50-gm. portions of each diced sample in a Waring blendor for 3 minutes with about 1 gm. of calcium carbonate and sufficient cold 2% aqueous sodium-chloride solution to make a total volume of 200 ml. The larger solid particles were removed by filtration through a gauze-backed cotton milk filter. The filtrates were held at 5° C., and the enzyme assays were usually made within 5–60 minutes. The enzymes were quite stable under these conditions, as shown by determinations of activity after the extracts had stood overnight. Preliminary results showed that the enzyme activities of (a) filtrates prepared as described, (b) unfiltered salt-carbonate suspensions, and (c) aqueous suspensions of the carrots were all about the same. However, filtrates of aqueous extractions exhibited only two-thirds of the total peroxidase and one-third of the total ascorbic-acid oxidase activity. Catalase was apparently completely soluble in water; the calcium carbonate served to stabilize it. Since peroxidase and ascorbic-acid oxidase apparently exhibit full activity, even when not in solution, it is probable that nearly complete extraction of these enzymes (and of catalase too, presumably) was obtained by the salt-carbonate procedure adopted. The activities obtained by this procedure were therefore designated enzyme content.

Enzyme assays were made on each of the duplicate extracts. A single run, involving at least three time intervals, was made for peroxidase, catalase, and ascorbic-acid oxidase; for phosphatase a single time period was used, but duplicate determinations at each of two dilutions of the extract were made. The enzyme-content values for the duplicate extracts generally did not differ by more than 10%.

PEROXIDASE.—A colorimetric procedure was used to determine the distribution of peroxidase in the tissue and a titrimetric procedure was used to determine the enzyme content of the peeled diced carrots.

The reagents for the colorimetric procedure were 10% guaiacol in 95% alcohol; 1 M acetate buffer (pH 5.6); and 0.75% hydrogen peroxide (1 ml. of 30% hydrogen peroxide diluted to 40 ml. with cold distilled water), which was prepared daily and kept at 5° C. when not in use. The reaction mixture consisted of 2.5 ml. of buffer, 1 ml. guaiacol, and sufficient water to make 50 ml. when the enzyme (usually 1–5 ml., depending on activity) and 1 ml. of hydrogen-peroxide

solution were added. The hydrogen peroxide was added last, after the mixture reached room temperature ($25°$ C.). The completed mixture was immediately stirred and a portion poured into a colorimeter tube for determination of the rate of color formation. The rate, which was linear at first, was determined with a Klett-Summerson colorimeter, using filter no. 42 (spectral range 400–465 mμ). The method correlated well with the titrimetric procedure. The activity, expressed as delta colorimeter reading per minute, may be converted to the peroxidase units defined below by multiplying by 2×10^{-5}.

The titrimetric peroxidase method was a modification of the BALLS and HALE (6) procedure. The significant changes were the use of guaiacol as the substrate instead of pyrogallol, the use of a lower pH occasioned by the change in substrate, and the addition of an excess of standard thiosulphate solution to the mixture in which the unused hydrogen peroxide liberated iodine from potassium iodide. As the iodine was liberated, it reacted with the thiosulphate. Thus it was possible to titrate with standard iodine to a sharp end point, even when assays were made on extracts containing considerable soluble starch (for example, extracts of blanched potatoes).

BALLS and HALE (6) showed that catalase did not give "apparent" peroxidase activity by their method, although the method is based on the measurement of the rate of disappearance of peroxidase. They reported that all the oxygen from catalase action (also molecular oxygen) combined with pyrogallol in such a way (apparently to form an organic peroxide) that it was determined by the analytical procedure, and therefore a net loss in peroxide owing to catalase action would not occur. It was also shown that the

oxygen-pyrogallol reaction product could serve as a source of peroxide for peroxidase. Because it is not sensitive to iron compounds (24), guaiacol was used in place of pyrogallol in our experiments; for the same reason guaiacol has been considered more reliable as a substrate for peroxidase tests made in connection with food dehydration. Since guaiacol does not combine with oxygen as readily as does pyrogallol, it was expected that catalase would interfere in the guaiacol-peroxidase method, but no interference was encountered. By use of the Warburg apparatus it was shown that the liberation of oxygen by carrot catalase was inhibited more than 95% by guaiacol. (Horse-liver catalase, however, was only 50% inhibited.) Also, the peroxidase activity of carrot extracts determined by the decrease in peroxide agreed with the activity determined colorimetrically by the increase in guaiacol oxidation product.[1] The values obtained were 1.80×10^{-3} and 1.86×10^{-3} units, respectively. It is evident that carrot catalase does not cause a significant rate of disappearance of peroxide from the reaction mixture and that the titrimetric-peroxidase values are valid. On the other hand, with a mixture of potato peroxidase and liver catalase, the rate of disappearance of peroxide was greater than the rate of appearance of color, as was expected from the finding that liver catalase activity was not completely inhibited by guaiacol under the peroxidase test conditions.

CATALASE.—The method of BALLS and HALE (5) was used, with the exception that the residual hydrogen peroxide was determined by the modification described in the section on peroxidase assay procedure.

[1] The factor for conversion of the colorimetric units to units based on moles of peroxide used was determined with a catalase-free peroxidase preparation.

ASCORBIC-ACID OXIDASE.—The rate of enzymic oxidation of ascorbic acid by carrot extracts was determined in a reaction mixture that contained oxalate and citrate to reduce the oxidation due to non-enzymic (heat-stable) substances. Under the conditions used, impurities (for example, traces of copper) in the distilled water, or in the chemicals used to prepare the extracts, did not affect the rate of oxidation of ascorbic acid appreciably. However, oxidation of ascorbic acid occurred with heated extracts at a rate of 5–15% of that obtained with the unheated extracts.[2] The results reported are therefore the differences between the rates found for the heated and for the unheated extracts.

The substrate was prepared daily by dissolving 20 mg. of ascorbic acid in 100 ml. of pH 5.6 buffer that was 0.2 M with respect to citrate and 0.01 M with respect to oxalate. The reaction mixture consisted of 5 ml. of substrate, the enzyme, and sufficient water to make a total volume of 11 ml. These were all at 25°, the assay temperature, before mixing. Immediately after thorough mixing, and at suitable intervals thereafter (5, 10, 15, and 20 minutes), 2-ml. aliquots of the mixture were removed and added to 3 ml. of 1.67% oxalic acid previously measured into a colorimeter tube. The oxalic acid stopped the enzyme action and stabilized residual ascorbic acid, which was determined within 2 hours. For the determination, 5 ml. of a solution of 2,6-dichlorophenol indophenol dye (90 mg. per liter of 0.01 M sodium acetate solution) was added to the colorimeter tube and the residual color intensity read uniformly, within 30 seconds, in a Klett-Summerson colorimeter with a no. 54 filter (spectral range 500–570 mμ). The colorimeter reading obtained after the dye was completely reduced by the addition of one drop of 0.1 N thiosulphate solution served as the blank value. Thiosulphate did not affect the reading obtained on samples to which no dye was added. From the net readings obtained and the previously determined relation of colorimeter reading to millimoles of ascorbic acid, the rate of disappearance of ascorbic acid from the reaction mixture was calculated in millimoles per minute. The rate obtained in a similar manner after heat inactivation of the enzyme was subtracted from that obtained with the active enzyme. The rate was approximately constant until half to two-thirds of the ascorbic acid was oxidized.

PHOSPHATASE.—The substrate, disodium phenyl phosphate, and the reagent (FOLIN's) used for determining the hydrolytic product, phenol, were the same as used for milk phosphatase by the milk industry (16). The assays were carried out at 47° C., the temperature used in the milk phosphatase test, and at pH 5.4, which is near the optimum for carrot phosphatase. The substrate was prepared by dissolving 1 gm. of disodium phenyl phosphate in one liter of 0.05 M acetate buffer, pH 5.4.

Ten ml. of substrate was placed in 25-ml. Erlenmeyer flasks and warmed to 47° C. in a water bath. One ml. of diluted extract (for carrots, 1 + 9 and 1 + 19 dilutions gave suitable activities) was added to the substrate, followed exactly 10 minutes later by 3 ml. of 2 N NaOH solution. The alkali served to stop the enzyme action and to furnish the alkali required for the phenol determination. Controls, or blanks on the carrot ex-

[2] With materials containing large amounts of ascorbic-acid oxidase, the rate of oxidation of ascorbic acid by the correspondingly small amounts of heated extract was negligible. For comparison, the ascorbic-acid oxidase activity of squashes is of the order of 100 times the activity of carrots.

tracts, were run by adding 3 ml. of 2 N NaOH solution to the warm substrate and then adding amounts of carrot extract equivalent to those used in the enzyme tests. Immediately after the addition of the alkali (or carrot extract in the case of the controls), the flasks were removed from the water bath, cooled to room temperature in cold water, and 4.5 ml. of diluted $(1 + 2)$ FOLIN's phenol reagent (9) added to each flask. The solutions were thoroughly mixed and the blue colors read after 2–4 minutes in a Klett-Summerson colorimeter, using filter no. 66 (spectral range 640–700 mμ). The phenol equivalent corresponding to the difference between the readings for the control and the test determination was read from a standard curve prepared from data obtained for known amounts of phenol.

ENZYME ACTIVITY UNITS.—One unit of peroxidase, ascorbic-acid oxidase, or phosphatase activity is the amount of enzyme that will transform to the reaction products one millimole of substrate per minute under the preceding assay conditions. The activities so expressed would correspond to the relative enzyme activities to be expected *in vivo*, except that the substrate concentrations are doubtless not the same as those present *in vivo*, and in the case of peroxidase and phosphatase the substrates are not the "natural" substrates. Nevertheless, it is felt that the activities expressed in these comparable units give in convenient form some idea of the order of magnitude of the potential relative activities of these enzymes *in vivo* and in any case are an index of the *in vitro* relative efficiency of enzyme preparations (17). The observed activities have not been converted to uniform temperature. Catalase was determined at 5°, peroxidase and ascorbic-acid oxidase at 25°, and phosphatase at 47° C. For catalase and phosphatase,

approximate values for the activities at 25° may be obtained by multiplying the reported catalase values by 1.9 and the reported phosphatase values by 0.4. For carrot peroxidase, one unit of activity as just defined was found by actual determination to be equal to about 200 purpurogallin units.

The usual first order k value (log base 10) in minutes^{-1} is used to express the catalase activity. Under the conditions used, $k \times 0.23 = \text{m}M$ of hydrogen peroxide decomposed per minute. The conversion factor (0.23 in this case) is directly proportional to the concentration of hydrogen peroxide, whereas k, of course, is independent of it.

Results

The outer tissue (cortex) of the carrots was removed by abrasive peeling in preparation for dehydration studies and for the various enzyme determinations. To determine whether variation in the amount of peeling would influence the results, the enzyme contents of the outer layer of tissue (about $\frac{1}{16}$-inch thick) and of a composite of the peeled portion were determined.

The extract prepared from the outer tissue (peel) contained about five times as much peroxidase, about the same amount of catalase, about 80% as much phosphatase, and about 60% as much ascorbic-acid oxidase as the extract prepared from the peeled material. Since the peel (as prepared) represented only 15–25% of the carrot, the catalase, ascorbic-acid oxidase, and phosphatase activities (but not peroxidase activity) in the extract of the peeled portion would be nearly independent of such variations in the amount of peeling. Also, the activity per gram for these enzymes would not differ by more than 10% from the activity per gram for whole unpeeled carrots.

A study of the distribution of peroxi-

dase in a sample of Long Imperator carrots showed that the enzyme content increased from the center to the outside. Thus, the xylem contained about 1.0, the cambium layer (plus some xylem and phloem) about 1.4, the inner part of the phloem about 2.4, and the cortex about 17 units per kilogram (fig. 1). It is estimated that the peroxidase content per kilogram of unpeeled carrot might be two to three times the value found for the peeled portion. Although the variations in the peroxidase values due to nonuniform peeling are probably small, this source of error must be kept in mind when interpreting the data.

The range of peroxidase content of the eleven varieties was about twofold for a particular harvest (fig. 2). The pattern of variation (that is, the relative varietal activities) was not uniform for all harvests, though the patterns are similar for the July and September harvests. The range of activities without respect to harvest was about 3.5-fold. The average values for the July and September harvests, which barely differed significantly, were about 20% higher than the values for the winter harvests, which did not differ significantly.

The range of catalase content of the different varieties was about twofold for the July and September harvests and about threefold for the winter harvests (fig. 2). The range without respect to harvest was about 4.5-fold. The data indicate that the season of the year rather than the age of the carrots (within the limits studied here) determines the level of catalase activity. Thus, the catalase values for all varieties are uniformly low for the January harvest, intermediate for the March harvest, and highest for the two summer harvests.

The range of ascorbic-acid oxidase content was about threefold for the March harvest but only about twofold

for the other harvests (fig. 3). In this respect ascorbic-acid oxidase was similar to peroxidase and catalase. However, the range without respect to harvest was about tenfold, in contrast with the 3.5- and 4.5-fold range for the other enzymes. The uniform increase in enzyme content for every variety in the order January, March, September, and July, of course indicates that environmental factors more or less uniformly influence the ascorbic-acid oxidase content.

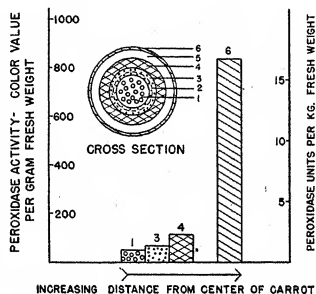The variations in the ascorbic-acid oxidase content for the two plantings

FIG. 1.—Distribution of peroxidase in carrots: 1, xylem; 3, cambium layer (plus some xylem and phloem); 4, inner portion of phloem; 6, outermost portion of phloem and pericyclic cork cambium (about $\frac{1}{32}$ inch thick). Numbers 2 and 5 designate, respectively, cambium layer and outer phloem adjacent to pericyclic tissues not assayed. Activities determined by colorimetric procedure.

were most interesting when compared with the ascorbic-acid values. Figure 3 shows that the ascorbic-acid content values varied considerably less from harvest to harvest than did the enzyme values. Nevertheless, when each planting is considered separately, the differences in ascorbic-acid content for the two harvests qualitatively paralleled the differences in enzyme content. As shown by the shaded portions in figure 3, both en-

zyme and acid are uniformly lower for the January than for the March harvest. A similar though inverse situation exists for the July and September harvests.
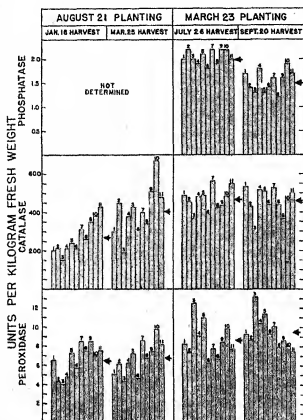

FIG. 2.—Peroxidase, catalase, and phosphatase values of carrots planted and harvested at different seasons (varieties given in table 1). Enzyme determinations for January 18 harvest were made on water extracts. To facilitate comparison of values for this harvest with later harvests in which salt extraction was employed, data for peroxidase have been calculated to expected salt value, using factor 1.5, which was found to be the ratio of salt extract values to water extract values. Catalase data required no correction. Arrows indicate average enzyme value for respective harvests.

with one exception. These aspects of the data indicate, as might be expected, a functional relationship between the ascorbic-acid oxidase and the ascorbic acid. Similarly, a functional relationship is indicated by the report of WOKES and ORGAN (26) that in tomatoes the highest concentrations of both ascorbic acid and ascorbic-acid oxidase are in the skins.

Phosphatase determinations were made on the March 23 planting only. The range of phosphatase content for the July harvest was 1.2-fold and for the September harvest was 1.6-fold. All varieties decreased in activity with age between the two harvests, the average decrease being 25%. Phosphatase resembled ascorbic-acid oxidase, in that the values for both enzymes decreased with age for the March 23 planting.

In addition to the work reported here, carrots were tested for catecholase and lipoxidase. The amounts of these two en-
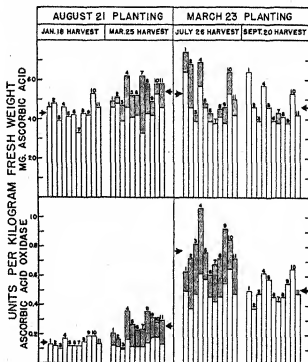

FIG. 3.—Ascorbic-acid oxidase and ascorbic-acid values of carrots planted and harvested at different seasons (varieties given in table 1). Ascorbic-acid oxidase data for January 18 harvest calculated from water-extract values to expected salt-extract values by factor 3.5 (see fig. 2 legend). Dotted areas indicate differences between the two harvests for each planting. Arrows indicate average value for respective harvests.

zymes in the peeled portion were insufficient to justify including them in quantitative work. The cortex, however, does contain readily measurable quantities of catecholase.

Discussion

The data have been reported on the fresh-weight basis. While for some purposes results expressed on a dry-weight basis are to be preferred, comparisons on the fresh-weight basis give better indexes of the relative concentrations *in vivo*. In order that activities may be calculated to a dry-weight basis, the moisture contents determined by drying the carrots

oxidase, and phosphatase, which were studied in this investigation, cannot be definitely stated. Catalase may be a scavenger enzyme that decomposes excess hydrogen peroxide (injurious amounts) formed by other enzyme systems (11, p. 20). Peroxidase may be involved in plant respiration, since it could utilize hydrogen peroxide or monosubstituted organic peroxides to oxidize fla-

TABLE 1

PERCENTAGE MOISTURE CONTENT OF ELEVEN VARIETIES OF CARROTS
PLANTED AND HARVESTED AT VARIOUS SEASONS

VARIETY NO.	VARIETY	PLANTED AUGUST 21, 1942			PLANTED MARCH 23, 1943		
		Harvested		Change (%)	Harvested		Change (%)
		Jan. 18	Mar. 25		July 26	Sept. 20	
1	Long Imperator....................	87.2	90.3	3.5	87.5	88.7	1.4
2	Regular Imperator................	87.2	89.8	3.0	88.0	88.9	1.0
3	M. B. no. 31.....................	87.5	90.7	3.7	87.4	88.2	0.9
4	No. 122.........................	88.7	90.7	2.0	88.8	89.6	0.9
5	R. C. Danvers....................	88.3	89.2	1.0	88.3	89.5	1.4
6	Nantes..........................	89.1	89.5	0.4	88.7	89.4	0.8
7	Chantes.........................	88.9	89.9	1.1	87.9	89.2	1.5
8	Improved Chantenay..............	88.0	89.4	1.6	88.0	89.7	1.9
9	R. C. Chantenay*................	88.8	89.3	0.6	89.2	90.3	1.2
10	R. C. Chantenay*................	88.9	88.8	−0.1	88.7	89.6	1.0
11	Short Top Shippers..............	88.9	89.4	0.6	87.9	89.7	2.0
	Average...............................	88.3	89.7	1.6	88.2	89.3	1.2

* Varieties 9 and 10 were not identical. No. 9 was the standard Red Core Chantenay type identified as lot pC3668 by the Associated Seed Growers, Inc.; no. 10 was their lot pC423627 of different origin, of somewhat different root type, and was considered outstanding for its interior color.

for 16 hours in vacuum at 70° C. are recorded in table 1. Relative values for enzyme and for ascorbic-acid content, on a dry-weight basis, would differ to some extent, but the indicated correlations would not be changed.

The importance of enzymes in metabolism of plants and animals is well recognized. The role that individual enzymes play in metabolism is indicated by their known characteristics, but definite establishment of their specific role is lacking. The possible physiological role of catalase, peroxidase, ascorbic-acid

vones, tyrosine, ascorbic acid (indirectly), and other tissue constituents. This view is given impetus by the findings that plants and fruits that lack polyphenol oxidase are usually rich in peroxidase (11, p. 157) and that mono- and polyphenolases are involved in plant respiration (4, 21). Ascorbic-acid oxidase, of course, may be involved in oxidation cycles also, since ascorbic acid can readily undergo reversible oxidation (15, 8). Phosphatase is only one of several enzymes that act on phosphate compounds, which are of importance in carbohydrate

metabolism (12, 13, 18, 7) and in the utilization of metabolic energy (10). It therefore seems likely that conditions leading to high metabolic rates would lead to high concentrations of enzymes that catalyze the formation or scission of phosphate bonds.

The metabolic rate doubtless varies with environmental conditions, physiological age, and variety. As judged by gross appearance, the physiological age for all harvests seemed to be about the same, in spite of the fact that the carrots were 4, 5, 6, and 7 months old when harvested. It therefore seems justified to attribute the differences in enzyme content chiefly to varietal and environmental differences.

Varietal differences in enzyme content are reflected by the values obtained for any single harvest. The maximum difference for a single harvest was 3.3-fold (ascorbic-acid oxidase, March harvest) and the minimum difference was 1.2-fold (phosphatase, July harvest). The individual enzyme-content pattern was not uniform for the four harvests, although there were some similarities. Thus, the catalase values for variety 3 were the lowest in all harvests, and the peroxidase values for variety 6 were consistently below varieties 5 and 7. On the other hand, the peroxidase values for variety 3 were the lowest for the first planting and the highest for the second. Speculations concerning these variations would seem to be of little value without additional data. Enzyme values that differed by less than 15% of their average were not considered significant.

The differences in enzyme values varied qualitatively with the environmental conditions of temperature and photoperiod. These conditions were probably best for growth at the time of the July harvest and decreased in order

for the September, March, and January harvests. The enzyme values were approximately in this order, as shown by the averages (indicated by arrows, figs. 2 and 3). The reversal of the order of the peroxidase values for July and September was an exception, but the difference was small, as were the differences for the other enzymes for these harvests. This is consistent with the smaller differences in the corresponding environmental conditions.

RUBIN and SPIRIDONOVA (22) suggested that a positive correlation between ascorbic acid and ascorbic-acid oxidase indicated that these substances played an active role in the tissue metabolism, and that a high vitamin content and a low enzyme content indicated that the tissue, with respect to ascorbic acid, served primarily as a storage place for the vitamin. Since the ascorbic-acid oxidase and the ascorbic-acid values for each planting varied qualitatively in the same direction, the postulate of RUBIN and SPIRIDONOVA would indicate that the vitamin plays a metabolic role in carrot roots and is not merely stored there.[3]

Summary

1. Two harvests (2 months apart) were made of eleven varieties of carrots planted in August and in March. The carrots were of prime quality when harvested. The peeled portions were assayed for catalase, peroxidase, ascorbic-acid oxidase, and (in the case of those planted in March) also for phosphatase.

2. The activities per gram of peeled tissue were about the same as for the whole carrot root, except for peroxidase, which in the samples tested was concentrated in the peel. With the exception of

[3] The low content of ascorbic acid (5–6 mg. per 100 gm.) in carrot roots compared with other plant tissue indicates that such roots are not a storage place for ascorbic acid.

peroxidase, which varied little in average activity for the eleven varieties, the highest activities were found for carrots harvested in July, followed in order by those harvested in September, March, and January. The highest average enzyme activities were thus obtained under the most favorable growing conditions of temperature and photoperiod. The differences in average enzyme activity appeared to reflect differences in the growing condition rather than in the age of the carrots (within the limits of the physiological age studied).

3. Within a single harvest the differences in enzyme content (that is, differences attributable to variety) ranged from 1.2-fold for phosphatase to 3.3-fold for ascorbic-acid oxidase, and averaged about twofold for the four enzymes studied. The ratios of the average enzyme contents for the July (best growing condition) and the January (poorest growing condition) harvests were 1.3 for peroxidase, 1.7 for catalase, and 5.5 for ascorbic-acid oxidase.

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CYSTOLITHS AND PLASMODESMATA IN BELOPERONE, FICUS, AND BOEHMERIA

FLORA MURRAY SCOTT

Introduction

Cystoliths, the name given by WEDDEL (31) to the stalked bodies described by MEYEN (14) in *Ficus elastica*, occur more or less abundantly in the Moraceae, Urticaceae, Acanthaceae, and numerous other families (28). For more than a century they have aroused the interest of morphologists (1, 2, 4, 6, 7, 8, 12, 14, 15, 16, 18, 19, 20, 24, 25, 26, 27, 31, 32, 33), physiologists (3, 5, 11, 29), and taxonomists (9, 22, 23, 28). The cystoliths in *Beloperone* are briefly described by HOBEIN (9), and by SCHACHT (25). They have been discussed under various names—Gummikeulen, glandes crystallines, corpuscules pédicellés, Traubenkörper, and as stalked papillate bodies, more or less ellipsoidal in outline, with a chalk-impregnated, stratified skeleton. In the careful illustrations in papers both early and recent, the cystolith or decalcified skeleton consistently appears suspended in bleak isolation within a cell, the lithocyst, which is entirely or almost entirely devoid of protoplasmic contents (for example, in the illustrations

by DE BARY (2) and in AJELLO's recent paper (1)). In contributions concerned mainly with the physiology of cystolith development, the protoplast of the lithocyst is not discussed in any detail. To the taxonomist, the value of cystoliths lies in their form and distribution, and the living protoplasts are again generally ignored. RADLKOFER (22), however, while discussing anatomical characters in relation to taxonomy, presumably became temporarily interested by the fundamental problem of cell differentiation, and comments briefly on the presence of sieve-fields in the lithocyst walls of *Momordica* (22). These, which today would be termed plasmodesmata, are sometimes interpreted as paths of intercellular communication. The general distribution of plasmodesmata, however, was not further recognized at this time (13).

That the outline of the cystolith—warty, papillate, or spiny—is conditioned by the activity of the protoplast of the lithocyst has perforce been accepted by previous observers, but any sug-

gested mechanism for this control has been left to the imagination. In the present paper it will be seen that, in *Beloperone californica*, the direction of the cytoplasmic strands within the lithocyst and the growth of the papillae which determine the outline of the cystolith are interdependent. In turn, the orientation of the intracellular protoplasmic strands is dependent on the distribution of plasmodesmata.

Examination of mature cystoliths in the classic types, *Ficus elastica* and *Boehmeria nivea*, reveals the same relation between cystolith papillae, cytoplasmic strands, and plasmodesmata. It may therefore be presumed that in other families where cystoliths occur, the form of the latter is determined, as in *Beloperone*, by the position of plasmodesmata in the cell wall and by the direction of the cytoplasmic strands of the lithocyst.

Material and methods

Beloperone californica, the sole member of the family Acanthaceae in southern California (17), is a rushlike, low-growing shrub common in the washes of the Colorado desert.

The tissues of leaf, stem, and root were examined as fresh material and after imbedding. In sections of fresh material, necessarily relatively thick, undamaged lithocysts were observed. The following stains and reagents proved useful: aniline blue with or without IKI, cotton blue, methylene blue, Janus green, neutral red, Ruthenium red, IKI, osmic acid, and Sudan III. Decalcification was effected slowly or rapidly with varying strengths of acetic, hydrochloric, or sulphuric acids. Pits and plasmodesmata were clearly seen on irrigation with IKI followed by careful addition of sulphuric acid of gradually increasing

strength (10% and upward). The techniques of LIVINGSTON and others in demonstrating plasmodesmata (10) confirmed the above observations.

Sections of imbedded material were cut at 6–25 μ. Some were stained with safranin and fast green, or crystal violet and erythrosin (10), and mounted in Clarite or Canada balsam. Others were treated for a short time (5–15 minutes in 5% H_2SO_4 in order to swell the walls slightly. After thorough washing they were stained with the reagents listed or with Delafield's haematoxylin and thereafter were mounted in glycerin.

Distribution of cystoliths was observed in leaves cleared in chloral hydrate. With sections of the leaves of *Ficus* and *Boehmeria*, anilin blue followed by IKI, Janus green, IKI followed by H_2SO_4, and Ruthenium red proved the most useful reagents.

Observations

In *Beloperone*, as in the Acanthaceae in general, lithocysts are distributed throughout the entire plant and may appear in any tissue except the xylem. They are first evident in the meristematic tissue of the shoot, differentiate early, and reach their maximum size in the primary internodes. The outer cortex of *Beloperone*, as in many photosynthetic stems, consists of alternating strands of chlorenchyma and collenchyma. Lithocysts differentiate along the outer and inner margins of the collenchymatous strands, along the inner face of the chlorenchyma, and occasionally within the chlorenchyma itself. They are most conspicuous in the parenchyma of the inner cortex, either in mid-parenchyma or adjacent to the starch sheath.

During secondary thickening, the rushlike shoots of *Beloperone* remain green, and cork formation is delayed un-

til the end of the first growing season. Cell division is maintained in the epidermal, cortical, and pericyclic tissues, which thus keep pace with the expansion of the axis during vascular differentiation. Secondary cystoliths may arise within the newly formed parenchymatous cells of this region. The first traces of phellogen are apparent in subepidermal cells external to the collenchymatous strands. Phellogen differentiation proceeds centripetally. The living collenchymatous cells lose their characteristic wall thickening and are converted into a meristematic tissue, the cork cambium or phellogen. When this ingrowing phellogen reaches the level of the inner cortex, differentiation next proceeds tangentially along the inner face of the chlorenchyma. The cylinder of phellogen at this stage is therefore fluted in outline when seen in transverse section. As cork formation becomes active, the outer tissues, chlorenchyma, hypodermis, and epidermis are cut off by the development of phellem. Occasional small cystoliths may arise in the phelloderm. The cork of the main axis and of the base of the older branches is rugose and stratified. Stratification is due to deposition of calcium carbonate in phellem and phelloderm, but here the chalk is laid down on the cell walls parietally, not on a supporting internal skeleton as in a cystolith. The lumen of these chalky cells may be entirely obliterated. In the deep cracks of the oldest bark, white, glistening larva-like particles are abundant—the cystoliths freed from the ruptured cells of the cortical tissues.

In the leaves, lithocysts are numerous in the lamina, particularly along the major and minor veins, a distribution readily observable in leaves cleared with chloral hydrate. They occur in the upper and lower palisade tissue of the ap-proximately bifacial leaves, and also in the central mesophyll and in the parenchyma above and below the larger veins. In the cortex of the petiole also, cystoliths are fairly numerous but reach their maximum density of distribution in the parenchyma of the leaf base.

In primary roots occasional small cystoliths are evident in the cortex. In the rugose cork of the older roots, however, no cystoliths are observable. Calcareous concretions, however, are present which are similar to those in the cork of the main stem.

In the shoot of *Beloperone*, in meristematic cells within $30\,\mu$ of the stem apex, minute crystals are visible in the vacuoles of certain cells. They are at the limit of visibility and are recognized by their brilliant refringence and somewhat angular outline, as seen in living material and in prepared slides (fig. 1). At the base of the second leaf primordium, and also beneath the ventral surface of the projecting leaf rudiment itself, similar but slightly larger crystals are observable. An extremely tenuous pellicle may be detected around the vacuole, and the trace of an equally delicate stalk is sooner or later evident (fig. 2). In slightly older cells the individual crystals are merged into one composite body (fig. 3). Within the vacuolating expanding lithocysts, growth of the cystolith continues. Stalk and skeleton now become clearly defined, the latter roughly isodiametric and papillate, a miniature of the mature structure. The papillae are connected to the cell wall by slender cytoplasmic strands (fig. 4). In a slightly older stage, decalcification reveals a faint stratification in the skeleton. The base of the latter, the stalk, and the stalk base in the cell wall resemble cutin or suberin in refringence, but fail to give a positive test with Sudan III (fig. 5).
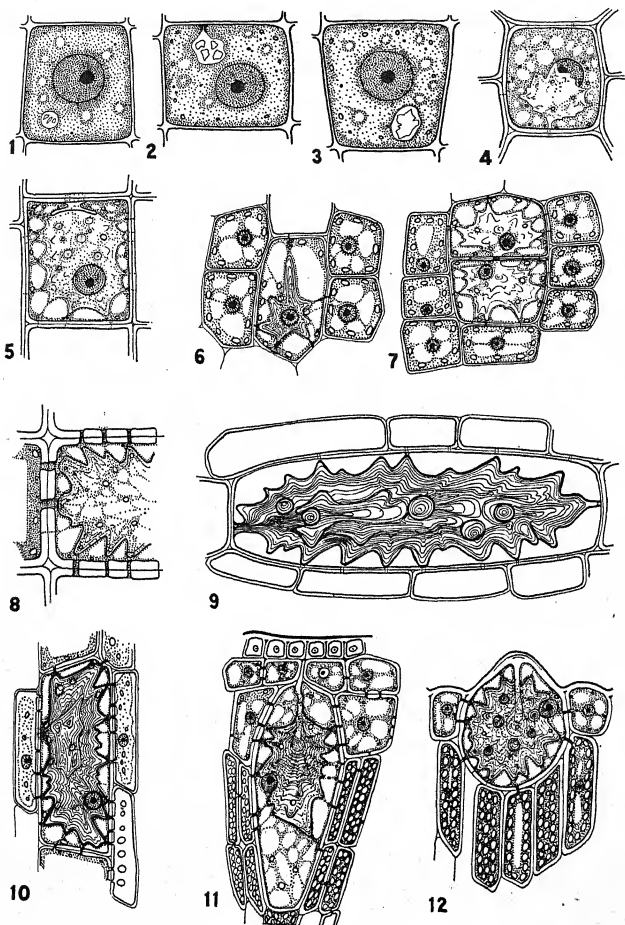
FIGS. 1–12.—Semidiagrammatic camera-lucida drawings: Figs. 1–10, *Beloperone californica:* fig. 11, *Ficus elastica;* fig. 12, *Boehmeria nivea.* Figs. 1–4, meristematic cells (8–12 μ) from longisections of stem apex (*1*, crystals in vacuole; *2*, showing number and size increase; *3, 4,* fusion, pellicle, stalk, and first traces of plasmodesmata). Figs. 5–7, vacuolating cells (15–40 μ) showing cytoplasmic strands radiating from cytoplasmic sheath of cystolith and anchoring in plasmodesmata in cell wall; fig. 7 shows paired cystoliths. Fig. 8, detail of plasmodesmata. Fig. 9, decalcified mature cell (140 × 50 μ). Fig. 10, mature lithocyst, laterally attached, with details of protoplast, nucleus, cytoplasm, chloroplasts, vacuoles, cystolith, and plasmodesmata in connection with adjacent cells. Fig. 11, cystolith from mature leaf; cytoplasmic strands and plasmodesmata similar to those in *Beloperone.* Fig. 12, cystolith from mature leaf; pattern of protoplast similar to that seen in *Beloperone* and *Ficus.*

In later stages of growth the details of the protoplasts of the lithocysts become increasingly clear. In contrast to the surrounding cells, the lithocysts enlarge but do not divide. The inclosed cystoliths more or less keep pace with lithocyst expansion and assume their characteristic spiny outline. The protuberances vary in length, but the longest extend almost to the now thickened and distinctly pitted cell wall. The position of the nucleus is variable and may be polar or central. Cytoplasm lines the cell wall and forms a sheath around the cystolith. From this sheath, across the vacuoles, cytoplasmic strands radiate outward toward an anchorage in the plasmodesmata, which are in general located within the pits of the cell wall. Chloroplasts are evident but are fewer in number and somewhat smaller in size than those of the photosynthetic cells. The stalk of the cystolith may be attached to horizontal or, less commonly, to lateral walls, and paired cystoliths are not infrequent (figs. 6, 7).

The cytoplasmic sheath is responsible for the deposition of the successive layers of the skeleton, cellulose and pectic materials, and calcium carbonate. The contour lines apparent on decalcification indicate differential activity in deposition in axial and in radial directions. The maximum width between growth lines occurs in general along the central axis, the minimum in the pointed spines. The period of most active growth of the cystolith coincides with the period of most active elongation of the internodes as a whole: The cystolith may lag behind the lithocyst in growth and may actually measure only about three-fourths of the latter in length. The tip of the cystolith, however, like all the rest of the papillae, is connected to the cell wall by a strand of cytoplasm. This strand is generally

very much wider and more conspicuous than the laterals, and the terminal plasmodesmata are correspondingly accentuated (figs. 6–10).

The cystoliths in the leaves of the classic types *Ficus elastica* and *Boehmeria nivea* are similar in fundamental structure to those of *Beloperone* (figs. 11, 12). In both, the surface projections of the skeleton are attached by cytoplasmic strands which are anchored in the plasmodesmata of the cell wall.

Discussion

Intercellular protoplasmic connections mentioned casually by RADLKOFER (22), as already mentioned, were first described in detail by TANGL (30) in endosperm tissue. Not until comparatively recently, however, has the ubiquitous occurrence of plasmodesmata been generally accepted (13). The numerous studies which followed MEYEN'S (14) initial observations centered mainly on the structure of the skeleton of the cystolith, with its stratification and more or less conspicuous radial striation. The lines of growth in the skeleton were attributed by RICHTER (24) to varying water content and molecular arrangement. This statement is sufficiently generalized to remain valid today. Radial striation has been explained in terms of cellulose-supporting strands (33) or to the presence of chalk-filled canals (24, 7).

Stratification of the skeleton of a cystolith is fundamentally similar to the lamination of a fiber wall, in that both are the product of protoplasmic activity. In a fiber the parietal cytoplasm is responsible for the deposition of wall material, while in a cystolith the central cytoplasmic sheath may be termed the active agent. In the differentiating lithocysts of *Beloperone*, vacuolating ex-

panding cells, the cytoplasmic sheath of the cystolith, and the parietal protoplasm are connected by cytoplasmic strands. These radiating strands are anchored in plasmodesmata. Skeletal materials are laid down tangentially in the body of the skeleton and radially in the spines. Radial striation is less accentuated in *Beloperone* than in *Ficus*. It is evident, however, that in all the cystoliths examined the radial lines result, as an optical effect, from the contrasted orientation of the skeletal particles.

In decalcified cystoliths the greater width between the lines of growth along the central axis is a measure of more active deposition of skeletal materials. The apical cytoplasmic strand, as has been noted, and the terminal plasmodesmata with which it is connected, may be wider and much more conspicuous than are the lateral strands. This may furnish one more piece of indirect evidence in regard to the function of plasmodesmata in the translocation of materials (13).

The earliest stages of cystolith development lie beyond the limits of microscopic definition. It seems reasonable to assume that plasmodesmata arise by the restriction of the generalized protoplasmic connections which exist in the walls separating the youngest protoplasts (13). One cytoplasmic strand in direct connection with such differentiating plasmodesmata may be considered responsible for the initiation of the cystolith stalk. The latter, as already seen, appears concurrently with the first traces of pellicle deposition.

In the walls of all lithocysts examined in various stages of growth, pits are more or less conspicuous, but no half-pits have

been observed. Symplastic growth in the walls of the expanding lithocyst thus appears to be indicated (21).

The fundamental question as to why materials reaching the cell are not directly deposited parietally on the cell wall, but are conveyed inward along cytoplasmic strands for the construction of a central skeleton, remains unanswered.

Summary

1. The occurrence and development of cystoliths in *Beloperone californica* is described.

2. Minute crystals appear in the vacuoles of certain meristematic cells of the stem apex. A pellicle surrounds the enlarging vacuole, the crystals become fused into one composite structure, and a tenuous stalk appears concurrently by which the structure is attached to the cell wall. *Beloperone* thus differs markedly from *Ficus elastica* (1) in the early stages of differentiation.

3. Within the lithocyst the cystolith is surrounded by a cytoplasmic sheath which is connected by radiating strands anchored to the plasmodesmata of the cell wall. The characteristic protuberances of the cystolith are laid down within these strands.

4. In the mature lithocysts of *Ficus elastica* and *Boehmeria nivea*, the pattern of the protoplast is similar to that of *Beloperone*.

5. The characteristic outline of cystoliths—warty, papillate, or spiny—is determined by the direction of the cytoplasmic strands of the protoplast, which in turn is dependent on the position of the plasmodesmata in the cell wall.

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HERBICIDAL ACTION OF 2,4-DICHLOROPHENOXYACETIC ACID ON SEVERAL SHRUBS, VINES, AND TREES

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Introduction

The use of 2,4-dichlorophenoxyacetic acid as an effective herbicide for bindweed, sow thistle, dandelion, plantain, and a number of other weeds has been reported (1, 2, 3, 4).

There are various woody plants which at times it is also desirable to destroy. Among these are Virginia creeper (*Psedera quinquefolia* L.); honeysuckle (*Lonicera canadensis* Marsh.); poison ivy (*Rhus toxicodendron* L.); chokecherry (*Prunus virginiana* L.), which harbors the X-disease of peaches; common juniper (*Juniperis communis* L.), the alternate host of cedar-apple rust; and hawthorn (*Craetegus* spp.), sumac (*Rhus typhina* L.), and elm (*Ulmus americana* L.), which infest pasture land. In addition, some woody plants such as poplar (*Populus nigra* L. var. *italica* Du Roi), plum (*Prunus domestica* L.), and pear (*Pyrus communis* L.) are troublesome because of a tendency to sucker from the stump. Furthermore, in forest operations it is sometimes necessary to thin the stand of trees. This paper presents results with 2,4-dichlorophenoxyacetic acid applied to these and other woody plants.

Material and methods

Inasmuch as previous work (1, 2) showed that concentrations of 2,4-dichlorophenoxyacetic acid as low as 1 part to 1000 parts of water were, in general, not so effective on several woody plants as on some herbaceous plants, means were devised for securing higher concentrations. By employing polyethylene glycol 200 and 400, Carbowax 1000 monostearate, and Carbowax 1540 distearate as carriers, concentrations could be increased to 2000 and 4000 p.p.m. Also, since the polyethylene glycols vary from liquid to solid state at ordinary temperature, formulations were made ranging from liquids of low viscosity (polyethylene glycol 400) to salves (Carbowax 1000 monostearate and 1540 distearate). The salve was prepared by mixing 10 gm. of Carbowax 1000 monostearate with 5 cc. of water. These concentrations were useful in applying the material in several ways—as water sprays to foliage, and as salves to stumps and cut surfaces. Various woody plants were thus treated.

Mean temperatures at the time of application and immediately afterward ranged from 28° to 85° F. Applications were made, both to plants which were in vigorous spring growth, with new shoots 2–6 inches in length, and also to plants in an older condition of summer growth.

An unusual feature of the season was an unseasonably warm period between April 11 and 14, followed by a prolonged cool period from April 15 to May 20, which was followed in late May and in June by temperature higher than seasonable (table 1).

Results

WATER SPRAYS APPLIED TO FOLIAGE IN APRIL

A water-soluble preparation secured from the Dow Chemical Company containing 70% 2,4-dichlorophenoxyacetic acid and 30% sodium bicarbonate at a concentration of 2000 p.p.m. (or 1400 p.p.m. of the acid) was applied to the foliage on April 11 and 20 of small plants

TABLE 1

MAXIMUM AND MINIMUM TEMPERATURES (° F.) DURING PART OF APRIL AND MAY, 1945 AT GENEVA, NEW YORK

APRIL	TEMPERATURE		MAY	TEMPERATURE	
	Maxi-mum	Mini-mum		Maxi-mum	Mini-mum
11........	81	49	1........	59	37
12........	82	53	2........	58	39
13........	85	51	3........	51	41
14........	84	45	4........	48	39
15........	54	29	5........	59	41
16........	56	34	6........	67	35
17........	70	51	7........	67	49
18........	64	35	8........	60	35
19........	54	32	9........	50	33
20........	55	34	10........	55	32
21........	50	32	11........	57	41
22........	44	30	12........	58	46
23........	54	28	13........	63	39
24........	55	30	14........	61	43
25........	50	52	15........	61	43
26........	61	48	16........	51	43
27........	57	35	17........	68	45
28........	41	33	18........	65	51
29........	47	37	19........	62	45
30........	59	37	20........	63	40

of hawthorn 2 feet and 4 feet tall, chokecherry 3 feet and 5 feet tall, elm trees 3 feet and 6 feet tall, juniper 6 feet tall, honeysuckle 3 feet and 5 feet tall, and poison ivy. These plants were growing on an abandoned nursery farm. For comparison, 2,4-dichlorophenoxyacetic acid (technical grade) was prepared in polyethylene glycol 200, using 2 parts of the acid to 5 parts of polyethylene glycol 200 to 1000 parts of water, and also applied to the foliage as a spray.

Plants of chokecherry, sumac, poison ivy, hawthorn, elm, and honeysuckle treated on April 11, when the temperature was 81° F., showed curvature of the new shoots and downward bending of the leaves within 5 hours of treatment. Warm weather prevailed for 4 days (table 1). Within 3 days the terminal growths of these plants were black and dead, the older leaves were mottled and edged with brown, and discolored spots appeared in the outer layers of the woody stems. All the plants treated on April 11 were dead above-ground within 3 weeks.

Plants of chokecherry, sumac, poison ivy, hawthorn, elm, and honeysuckle treated on April 20, when the temperature was 55° F. followed by a prolonged cool spell (table 1), responded much more slowly than plants treated during the earlier warm period. Only slight curvature of the new shoots was noted even 2 days after treatment. The plants appeared not to change much in appearance until after the onset of warm weather in June, when the treated plants of chokecherry (fig. 1), sumac (fig. 2), hawthorn, elm, and honeysuckle died. Only 50% of the poison-ivy plants were killed by this treatment, but many of the plants not killed were in a shaded area.

Juniper was also treated on April 11 and 20, but the plants showed no visible response. Two additional sprays at 4000 p.p.m. were applied to the juniper plants but again without apparent effect.

APPLICATIONS AS POWDER AND SALVES TO CUT SURFACES

The water-soluble powder from the Dow Chemical Company and the salves (polyethylene glycol 200 and Carbowax 1000 monostearate) containing 2,4-dichlorophenoxyacetic acid at a concentration of 20% were applied to the cut surface of suckering stumps of mature pear, plum, hawthorn, and poplar trees which had been cut the previous year. Fresh cuts were made with a hatchet and

the cut surfaces covered with the salve. When the powder was used, enough was applied so that the wound was entirely covered. The suckers on all treated stumps were affected within a horizontal radius of 4 feet, and they died following a period of typical stem curvature and browning of the leaves. The powder was slower in action than were the salves.

Salves and powder of the same material were applied on April 11 and on May 2 to young and mature trees of hawthorn, chokecherry, peach (*Prunus persica* Batsch.), poplar, pine (*Pinus strobus* L.), spruce (*Picea canadensis* Mill.), ash (*Fraxinus americana* L.), elm, and willow (*Salix nigra* Marsh.) by wounding the stem through to the wood with a hatchet and applying the materials to the cut surfaces. Cuts were made at various heights, both completely and partially encircling the trunks. In addition, holes were bored into the trunks and filled with the chemicals.

Treated plants responded generally by typical curvatures of new shoot growth within 2 days after treatment but varied otherwise in the degree and type of response. For example, leaves at the top of 40-foot poplar trees showed downward bending of the petioles within 2 days. Progressive browning and drying followed, so that within 1 month the leaves at the tops of the trees were dead, and the leaves on the lower parts of the trees were becoming twisted, chlorotic, and brown. At this time the bark immediately above the points of application was split longitudinally for distances of more than 6 feet, caused by proliferation in the region outside the cambium, and forming a layer of very soft, spongy new tissue $\frac{5}{8}$ inch in thickness (fig. 3). The bark immediately below the points of application was affected downward for a distance of approximately 2 feet. Lat-

eral transfer of the acid around the trunk was evident but not pronounced.

In the case of peach trees, not only were flowers and leaves distorted, finally becoming brown and dry, but also the limbs and branches exuded gum freely
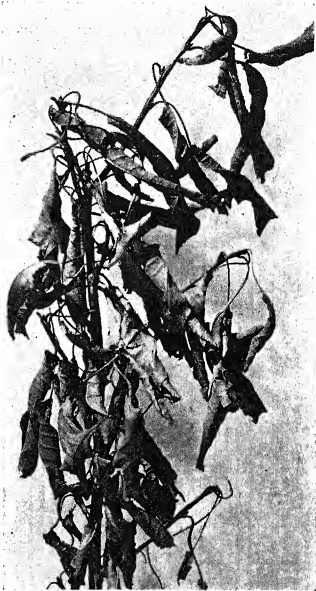

Fig. 1.—Dead and withered chokecherry plant following treatment with 2,4-dichlorophenoxyacetic acid.

from many small discolored areas (fig. 4), and the trees were completely dead within 60 days after application.

In the case of pine trees, the new terminal growth became severely twisted, the young cones became pendant, the bark split, and gum exuded from large

patches on the branches and trunks, which had a water-soaked appearance. Elm, hawthorn, ash, and chokecherry showed typical curvatures, browning and necrosis of leaves, followed by death of the entire tree. The effect of the material traveled upward in the tree more rapidly than downward, and the lateral movement within the plant was slower than upward or downward; nevertheless, there was some lateral movement. Young trees were more responsive than older ones, and the elm tree was the most responsive of all plants tested.
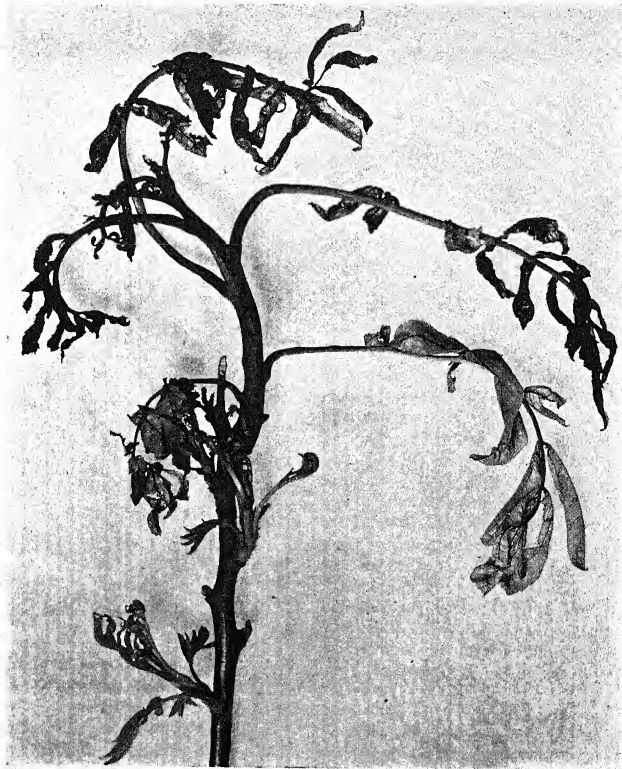
FIG. 2.—Blackened tips of sumac killed by water spray of 2,4-dichlorophenoxyacetic acid

WATER SPRAY APPLIED TO FOLIAGE
IN JUNE

On June 14, plants of Virginia creeper were sprayed with a solution containing 1200 p.p.m. of 2,4-dichlorophenoxy-
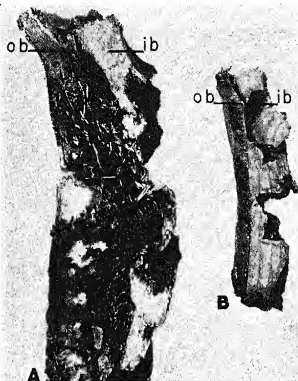

FIG. 3.—Effect of salves of 2,4-dichlorophenoxyacetic acid, applied to girdling wounds on poplar trees: *A*, longisection from treated tree, showing enlarged, proliferated, and decaying region of inner bark; *B*, same from untreated tree, showing normal spring growth. (*ob*, outer bark; *ib*, inner bark.) Sections taken 3 feet above treatment.

acetic acid (technical grade) in Carbowax 1500. The creeper had formed a mat of foliage in a cherry orchard, the plants being about 6 inches tall when sprayed. An area of 400 sq. ft. was treated. The spray, heated to approximately 110° F., was applied on a warm day, when the temperature was 85° F. Twenty-four hours after treatment the plants were greatly distorted and twisted. After 10 days all treated plants were dead back to the main stem. After 1 month some new shoots, about 3% of the

original cover, had reappeared and were making feeble growth.

On June 14, plants of wild grape (*Vitis vulpina* L.), growing near the cherry orchard previously mentioned, were sprayed with 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. Within 24 hours the leaves were twisted and distorted, and within 10 days the entire above-ground parts of the plants were dead.

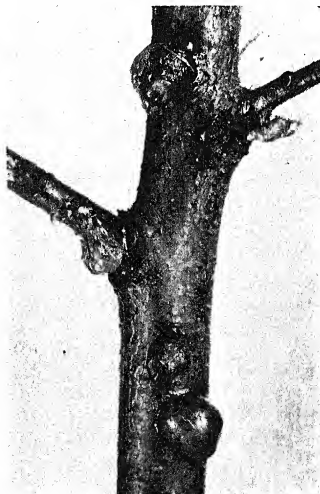On June 14, five clumps of willow trees about 10 feet high were sprayed

FIG. 4.—Portion of peach branch showing exudation of gum following treatment with 2,4-dichlorophenoxyacetic acid to girdling wounds in trunk. Section taken 6 feet above treatment.

with the water-soluble salt of the acid at 1000 p.p.m., and five clumps were sprayed at 2000 p.p.m. Within 2 days the leaves on all treated plants showed black-

ening along the midrib. The blackening was pronounced and very different from the type occurring in other plants. The leaves were ashy black as though seared by fire. Within 2 weeks the entire tops of the clumps were dead, and after 6 weeks no new shoots appeared from the roots. The response of the clumps to the spray at 2000 p.p.m. was more pronounced and more rapid than at 1000 p.p.m. In both treatments the plants died.

On June 14, twelve plants of chokecherry 6 feet tall, twelve plants of honeysuckle 5 feet tall, and three plants of hawthorn 4 feet tall were sprayed with the commercial salt of 2,4-dichlorophenoxyacetic acid at 2000 p.p.m. Within 10 days all but two plants of the chokecherry and all the honeysuckle plants were dead. The chokecherry plants that were not killed by the treatment were growing in a heavily shaded area. The hawthorn was affected, the growing points being blackened, but the plants did not die.

Discussion

Shrubs, vines, and trees can be killed by the use of 2,4-dichlorophenoxyacetic acid. Effective herbicidal results were secured with water sprays containing 2000 p.p.m. of the water-soluble preparation or with 2,4-dichlorophenoxyacetic acid carried in polyethylene glycol 200. In general, plants which had just broken dormancy in the spring and were making new growth were more sensitive to treatment than plants with fully developed foliage. Plants treated during warm weather (80°–85° F.) followed by a continued warm period, which favors active growth, responded much more quickly and more completely than did plants treated in cool weather (below 50° F.).

The elm was the most sensitive of the trees treated. Its long system of uninterrupted vessels perhaps aids in the rapid transport of the material throughout the plant. Grape and Virginia creeper were readily killed by treatment with the acid, but poison ivy seemed more resistant. Unless optimum conditions prevailed, a complete kill of poison ivy was not obtained.

Plants that were in a shaded area responded much more slowly than did plants exposed to direct sunlight. In a few cases, chokecherry trees in a heavily shaded area were not killed by treatment.

In treatments involving wounding of the bark, the most effective was complete girdling of the trunk and application of high concentrations in the form of salves directly in the notches formed by the girdling. For equal effects on large trees, greater amounts of the material were required than for smaller trees, indicating that there may be a quantitative relationship between the material and the response. The activation of the region outside the cambium and the increase in gum flow are of special interest, suggesting the possibilities of trials where increased bark and gum flow are desired, as in quinine and turpentine.

Summary

1. Experiments were conducted in the immediate vicinity of Geneva, New York, between April 20 and July 15, 1945, using several formulations of 2,4-dichlorophenoxyacetic acid as a herbicide applied as a water spray at concentrations of 1000 and 2000 p.p.m. to certain shrubs, vines, and trees. Concentrated salves of these materials were also applied to cut surfaces.

2. Applications as a foliage spray at 2000 p.p.m. during a warm period (80°–85° F.) in early April at the time of leaf emergence resulted in death of chokecherry, honeysuckle, poison ivy, sumac,

hawthorn, and elm after 3 weeks. No effect was observed on juniper.

3. Applications as a foliage spray at 2000 p.p.m. during a cool period (below 50° F.) in late April resulted in marked reduction in response. Death of sumac, chokecherry, honeysuckle, hawthorn, and elm did not occur until a warm sunny period in June. Fifty per cent of the poison ivy treated in late April completely recovered. No effect was observed on juniper.

4. Application to cut surfaces in early spring in the form of concentrated salves resulted in typical curvatures, browning and necrosis of leaves, exudation of gum, proliferation of the inner bark, and death of the entire plant, provided sufficient active material was used on the cut surfaces.

5. Application as a water spray in June at 1000 and 2000 p.p.m. resulted in death of the above-ground parts of Virginia creeper, grape, willow, chokecherry, and honeysuckle.

6. Application to hawthorn in June, when the leaves were fully expanded and mature, resulted in blackening of the growing tips but not death of the entire plants.

Grateful appreciation is expressed to ROBERT CARLSON and BARBARA IMHOFE of the New York State Agricultural Experiment Station for generous assistance in the course of this work.

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INFLUENCE OF CARBOHYDRATE AND NITRATE–NITROGEN NUTRITION ON DEVELOPMENT OF HYPOCOTYLEDONARY BUDS IN FLAX[1]

VIRGINIA EGGERS

Introduction

BURNS and HEDDEN (3) investigated conditions influencing hypocotyledonary bud initiation and shoot development in

Linum usitatissimum. They made tests with seedling plants 2–3 cm. high, decapitated immediately below the cotyledons, to determine the influence of moisture, temperature, age, gravity, and light on bud initiation and development. It was concluded that the time of bud initiation and the number of buds developed are dependent on light, moisture, and heat. Young hypocotyls are capable of developing more buds and in a shorter

time than old ones. The base of the hypocotyl of old plants is predisposed to bud production. The position of buds is due indirectly to light and directly to the movement of materials in the plants; good vegetative conditions are best for production of buds on the hypocotyl. Later, ADAMS (1) noted the influence of moisture on increasing hypocotyledonary bud and shoot development in flax.

The object of the present study was the determination of the influence of carbohydrates or the opportunity of their synthesis and the nitrate-nitrogen supply in the nutrient medium on the initiation and development of buds on the hypocotyl of flax after decapitation.

the control plants grown in soil and for the entire plantings of May 5 and 20, 1936.

The hypocotyls were cut at right angles to the axis when the cotyledons were well expanded and the epicotyl was beginning to elongate. The time necessary to reach this stage varied with the temperature. As a consequence, decapitations were made 11, 8, 6, and (in one case) 2 days after the plants appeared above the substrate. In one planting the hypocotyls were cut at three levels: (a) directly below the cotyledons, (b) halfway between them and the soil line, and (c) three-fourths of the way down the hypocotyl. As bud forma-

TABLE 1

CONSTITUTION OF (A) +N AND (D) −N SOLUTIONS (CC.)

Solution	Distilled H₂O	MgSO₄	KH₂PO₄	Ca(NO₃)₂	CaCl₂
A...............	67,490	630	630	1250	....
D...............	68,110	630	630	....	630

Material and methods

The Bison variety of flax was used. The work was carried on from October, 1934, to June, 1936, in the greenhouses of the University of Chicago. Both large and small growing rooms were used; the former proved more satisfactory because the humidity could be maintained at a higher level and the temperature was more nearly even. High temperatures resulted in rapid growth, but the plants were spindly. At first, temperatures of 18°–21° C. were tried, but these proved to be too high for development of sturdy plants, and other experiments were run at 15° and later at 10° C. Plants were grown in sterilized no. 3 quartz sand in glazed earthenware pots with bottom drainage. Unglazed flower pots were used for all

tion took place regardless of the place of cutting, this practice was discontinued, and in all further experiments the cut was made directly below the cotyledons but at sufficient distance to remove the cotyledonary node, thus guarding against regeneration from remnants of cotyledonary buds.

To study the effect of carbohydrate and nitrate-nitrogen supply on the development of hypocotyledonary buds, +N and −N nutrient solutions (solutions A and D, respectively) were applied to sand cultures once each day, at the rate of about 0.5 liter per pot. Additional water was not necessary at any time. The constitution of the solutions is given in table 1.

Occasionally traces of ferric phosphate

(FePO$_4$) were added to the daily portion of solution. In some experiments the $+N$ solution was diluted 2 parts of $+N$ with 1 part of $-N$ solution, and in others 1 part of $+N$ with 2 parts of $-N$ solution. These are designated as diluted nitrogen solutions B and C, respectively.

ARRHENIUS (2) reported that flax gives the highest yield at pH 6 and 4. CHIZHEVSKAIA (4) reported that pH 5, 6, and 7 are favorable. The planting of December 19, 1934, was treated so that plants received $+N$ and $-N$ solutions with pH 4 and 6. The only difference was a slightly greater number of buds developed by the plants receiving solutions with pH 6. In subsequent plantings the pH was adjusted to 6, except those of May 7, 1936, and later when the solutions were pH 5.

Several experiments were set up to vary the carbohydrate synthesis by varying the amount of light and so to test its effects upon hypocotyledonary bud formation. As the investigation covered a period of 17 months, the amount of natural light available was different for each planting. Artificial light from 1000-watt Mazda lamps in metal reflectors was added during certain seasons and natural light withheld at others in order to bring about a difference in amount of light received between experimental and control plants.

Observations

A difference in vegetative growth was noted between plants receiving the full amount of nitrogen (solution A) and those receiving less nitrogen (solutions B, C, D). There was a decided difference between the plants receiving solution A and those receiving solution D, and the plants receiving solution B were more thrifty than those receiving solution C.

There was positive correlation between pigmentation, succulence, height, and number of leaves, flowers, and seed-balls produced and the amount of nitrogen applied. While the average height of the soil-grown control plants was intermediate between that of the $+N$ and $-N$ plants, their sturdiness at all times was superior to both the $+N$ and $-N$ plants. Possibly this is due to an increased development of fibers and xylem.

Figure 1 shows $+N$, $-N$, and control plants 3, 15, 23, and 105 days old. In 3-days-old plants the main difference was slightly greater size of the $+N$ plants and a tendency to curled cotyledons of the $-N$ plants. At fifteen days, differences in size (height, and area and number of leaves) were even more pronounced, and in addition pigmentation differences were evident. The $-N$ plants were decidedly yellow green, with yellow cotyledons and a reddish cast of the hypocotyl owing to the increased amount of anthocyanin characteristic of plants high in carbohydrates and low in nitrates. At 23 days the $-N$ plants were severely stunted while the control plants were sturdy. The $+N$ plants were much taller than the controls but were so succulent that they did not stand upright without support. Buds in the cotyledonary axils had in many cases developed into shoots half as long as the primary axis. In plants a little older than these, which had assumed a horizontal position, buds developed in the axil of every leaf of the main axis, a decided departure from the usual habit of the flax plant. Some of these lateral shoots were 3 inches long. In other plantings, buds appeared in the axils of leaves of upright plants. In plants 105 days old the heights were as follows: controls 23 cm., $+N$ plants 43 cm., and $-N$ plants 17.5 cm. Both controls and $+N$ plants produced nu-

merous flowers, while the $-N$ plants altogether produced only three.

The amount of nitrogen in the solution also influenced formation and development of adventitious buds by decapitated hypocotyls. This difference manifested itself in the number of plants producing buds, the number of buds produced, and the rate of development of the newly formed buds (table 2; fig. 2).

light. The plants which received added light were taller, more plants produced buds, and the number of buds per plant was greater. The influence of light on the time of bud formation was not so clear in this experiment as in later trials.

In the planting started during February, light was varied by shading the plants with large boxes so that only diffuse light entered from the space left at
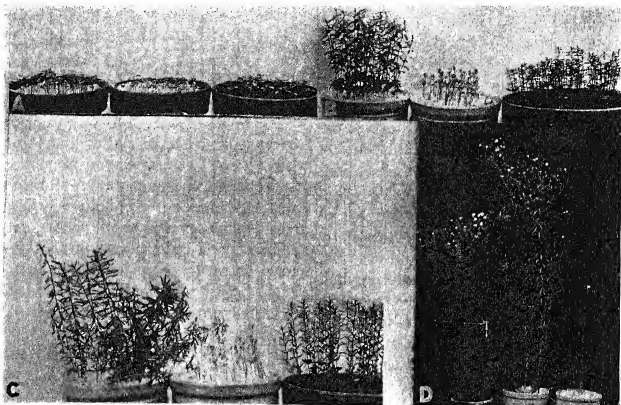

FIG. 1.—Influence of nitrate-nitrogen nutrition on growth. Reading from left to right: A, $+N$, $-N$, and control plants 3 days old.' B, $+N$, $-N$, and controls 15 days old. C, $+N$, $-N$, and controls 23 days old. D, controls, $+N$, and $-N$ plants 105 days old.

The first planting which received a controlled amount of light was started in December, 1934, when the days were short and the sky overcast most of the time. The plants were divided into two lots, half receiving no light and the other half receiving 4 hours of added light, starting at sunset. Mazda lamps, 1000-watt, in metal reflectors were placed about 20 inches above the top of the pots and spaced so that all plants received as nearly as possible an equal amount of

the bottom for ventilation. All the plants were left uncovered on overcast days, while half of them were covered about four-fifths of the time that the sun shone. With this amount of light the plants were maintained in fairly good condition. The plants which received the full amount of sun regenerated buds 6-10 days earlier than the shaded plants. These findings are in harmony with those of BURNS and HEDDEN (3).

In March, when the sunlight was more

intense and the period of light longer, this shading experiment was repeated. In this trial the four nutrient solutions A, B, C, and D were used. It is evident (table 2) that applications of nitrate nitrogen and abundant sunlight in combination are most favorable for bud regeneration. Plants receiving the full amount of sunlight produced buds 6–8 days earlier than the shaded plants. The fully illuminated lots also had a greater number of plants with adventitious buds and more buds per plant.

To test the effect of one-sided illumination, pots of plants were placed in boxes open only on one side, toward the west. The boxes fitted the pots closely, so that little light reached the plants from the side. Of the total number of buds

near the soil line and along the illuminated surface, whether this was the true upper surface as established by the horizontal position of the plants or the lower surface which had become exposed to the light because of the upward curving of the tips. These findings corroborate those of BURNS and HEDDEN.
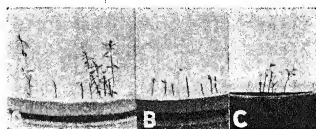

FIG. 2.—Decapitated hypocotyls showing influence of nitrate-nitrogen nutrition on growth made by buds during 19 days following decapitation. *A*, +N solution. *B*, −N solution. *C*, soil-grown control.

TABLE 2

HYPOCOTYLEDONARY BUD DEVELOPMENT IN RELATION TO LIGHT AND NUTRITION

	SHADED					LIGHTED				
	Soil (control)	Sand				Soil (control)	Sand			
		A	B	C	D		A	B	C	D
Total no. buds..............	7	14	8	5	4	38	31	24	13	2
No. plants with adventitious buds*...................	3	5	5	3	2	10	9	8	5	2
Average no. buds per plant with buds..................	2.3	2.8	1.6	1.6	2.0	3.8	3.4	3.0	2.6	1.0
Plants with adventitious buds (%)......................	30	50	50	30	20	100	90	80	50	20

* Ten plants in each lot.

developed, 50% were on the west or lighted side, 5% on the east or shaded side, 10% on the north, and 35% on the south.

To test the effect of one-sided illumination by another method, 10-days-old newly decapitated plants in small pots were placed horizontally. The tips of these hypocotyls turned upward toward the source of light. They produced buds

Discussion

From these results it is apparent that the nutritional status of the plant has a decided effect on the time and frequency of bud formation and development in decapitated hypocotyls of flax.

As in any meristematic region, a supply of nitrates and carbohydrates for the building of organic compounds was necessary for the growth of these adventi-

tious buds. A high carbohydrate supply, found in the plants receiving the full amount of sunlight, resulted in earlier development of buds (to the stage visible to the unaided eye) than in plants having a low carbohydrate supply owing to shading. A greater number of these plants produced buds, and their average number per plant was greater than in low carbohydrate plants. Within the two groups of plants, one high in carbohydrates and one low, a variation in the nitrate-nitrogen content of the nutrient solution had a direct relation to the num-

ber of plants which produced buds and the number of buds developed. In the shaded plants with restricted carbohydrate supply there was less production of buds owing to decreased amounts of nitrate nitrogen in the nutrient solution than in plants with a larger amount of carbohydrate. Under the conditions of the experiments, the maximum supply of carbohydrate and nitrate nitrogen resulted in maximum bud production. Minimum bud production was in plants high in carbohydrates but low in nitrate nitrogen.

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CYTOLOGICAL EFFECTS OF SULFANILAMIDE ON ALLIUM CEPA

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Introduction

Bulbs of *Allium cepa* with young roots were immersed in a 0.5% aqueous solution of sulfanilamide (18°-20° C.) for 48 hours. As a control and for purposes of comparison, other bulbs were immersed in a 0.1% aqueous solution of colchicine for the same period. At various intervals during treatment, and during 48 hours of recovery in tap water, root tips were cut and fixed in the Craf fixative or in acetic alcohol. The former material was imbedded in paraffin, sectioned at 10 μ, and stained with iron haematoxylin; the latter was prepared according to the acetic-orcein squash method.

Observations

During treatment with sulfanilamide the root grows very little in length. A

slight swelling, much less than that produced by colchicine, usually appears in the region of elongation. After 3 hours of treatment, sulfanilamide apparently has no effect on the prophase chromosomes—except possibly to bring out more clearly the division between chromatids. Cells which were in metaphase or anaphase at the onset of treatment with sulfanilamide complete the nuclear division but do not form a cell plate. Cells which were in prophase during treatment are blocked at metaphase, owing to failure of spindle formation. The metaphase chromosomes become shorter and thicker, forming a scattered group which, with few exceptions, will revert to a single tetraploid nucleus. Cells treated in early anaphase may form a reversion figure similar to the one shown in figure j, which would prob-

ably have given rise to a bridge-binucleate cell. Since sulfanilamide prevents the formation of a cell wall between daughter nuclei, cells in late anaphase or in telophase usually form binucleate cells. Three hours after the beginning of treatment there are many blocked metaphases with scattered diplochromosomes; some are more highly contracted than others, but all are shorter and thicker than normal metaphase chromosomes. The division of the SA-region is usually delayed until reversion begins, but at times the diplochromosomes divide earlier and form the tetraploid number of single, scattered chromosomes.

After 8 hours of treatment, diploid, blocked metaphases were abundant; some of them had super-contracted diplochromosomes scattered in the nuclear region or throughout the cell (figs. b, h). Tetraploid blocked metaphases were also present, but only rarely. Although the arms of the diplochromosomes are usually parallel, they occasionally diverge, forming X-shaped figures. Reversion figures may be regular in shape, or irregular when formed of scattered reverting chromosomes.

After 12 hours of treatment there is a decrease in the relative number of prophases with long thin chromosomes. The diplochromosomes of blocked metaphases are at times very short, with the arms still parallel to each other (figs. d, e). Micronuclei are found in some of the resting cells which have undergone reversion. After 28 hours, reversion stages in uninucleate and binucleate cells are abundant. Some prophases and blocked metaphases are also present. After 48 hours, divisions are scarce. There are a few blocked metaphases with scattered diplochromosomes.

Root tips treated with sulfanilamide for 48 hours and then placed in tap water for 48 hours recovered completely.

Such tips showed multinucleate cells (fig. m), resting cells of about twice the normal size, and some tetraploid mitotic figures (fig. p). These findings are in agreement with TRAUB's (4) report on root tips recovering from treatment with sulfanilamide. These abnormal types constitute less than 1% of the cells of a fully recovered root tip, and the majority of mitotic figures are those of normal diploid cells.

Discussion

During treatment with colchicine, X-shaped diplochromosomes with undivided SA-regions occur frequently, whereas under the influence of sulfanilamide the chromatids usually remain parallel to one another until a separation at the SA-region occurs (figs. a–g). This parallel position suggests the presence of a viscous matrix holding the arms of the chromatids together. Only rarely in sulfanilamide-treated material are the highly contracted chromosomes arranged around an achromatic sphere of apparently unorganized spindle substance (fig. l), as described by BERGER and WITKUS (1) in material treated with colchicine.

Figures c and f seem to indicate that reversion can take place even before the SA-region has divided. The physical appearance of the chromosomes indicates the onset of reversion to the resting stage, yet there is no apparent evidence that the SA-region has divided. This point of view is in accord with the description by HAWKES (2) of reversion stages under the influence of colchicine. He holds, in opposition to LEVAN (3), that the more common type of reversion is that in which the SA-region has not yet divided, and that the division takes place either in the resting stage or during the early stages of the following prophase. The paired condition of mid-

prophase chromosomes (fig. o) found after 48 hours of recovery after sulfanilamide treatment indicates that the cell became tetraploid at the preceding division and that the division of the SA-region had been delayed at least until the beginning of the reversion process.

Multinucleate cells and cells with micronuclei are due to the fact that at times scattered groups of chromosomes revert to a number of nuclei (fig. m) or that one or two chromosomes return to the resting stage independently of the main nucleus (figs. h, k, m, n).

Sulfanilamide in the concentrations used in this experiment inhibited the entrance of cells into mitosis. On the other hand, colchicine in concentrations of 0.1% did not prevent the onset of mitosis. In fact, an abundance of tetraploid figures during the forty-eighth hour of colchicine treatment indicated that the mitotic cycle can be fully completed and repeated under the influence of colchicine.

Cells recovering from colchicine treatment present a greater variety of abnormalities than those recovering from sulfanilamide treatment. Colchicine, in addition to multinucleate cells, cells with micronuclei, and tetraploid figures, causes high degrees of polyploidy, that is, $8n$, $16n$, and multipolar figures (3). In sulfanilamide material there were no multipolar figures, and the highest degree of polyploidy found was $4n$.

Summary

Sulfanilamide, like colchicine, inactivates the spindle mechanism and delays division of the spindle attachment region. Both sulfanilamide and colchicine induce polyploidy, but colchicine is more effective since it results in a greater percentage of polyploid cells and a higher degree of polyploidy. Sulfanilamide (0.5%), unlike colchicine (0.1%), inhibits the entrance of cells into mitosis. Colchicine results in multipolar mitosis; sulfanilamide does not.

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PLATE I

Figs. a–p.—Effects of sulfanilamide on cell division. All cells treated with 0.5% sulfanilamide and stained with acetic-orcein. ×1000. Fig. a, prometaphase, 12 hours' treatment. Fig. b, scattered diplochromosomes, highly contracted, 8 hours. Fig. c, diplochromosomes in very early reversion, 12 hours. Figs. d, e, diplochromosomes, very highly contracted, 12 hours. Fig. f, diplochromosomes, very early reversion stage prior to separation at SA-region, 12 hours. Fig. g, scattered single chromosomes just separated at SA-region, 12 hours. Fig. h, tetraploid number of single chromosomes in very early reversion, 8 hours. Fig. i, tetraploid number of single chromosomes in very early reversion, 12 hours. Figs. j, k, reversion phase, 6 hours. Fig. l, reversion phase, 24 hours. Fig. m, multinucleate cell, 48 hours' recovery after 48 hours of treatment. Fig. n, diploid metaphase with chromosome fragments, 48 hours' recovery after 48 hours of treatment. Fig. o, tetraploid prophase, with conspicuously paired chromosomes, 48 hours' recovery after 48 hours of treatment. Fig. p, tetraploid anaphase, 48 hours' recovery after 48 hours of treatment.

PLATE I
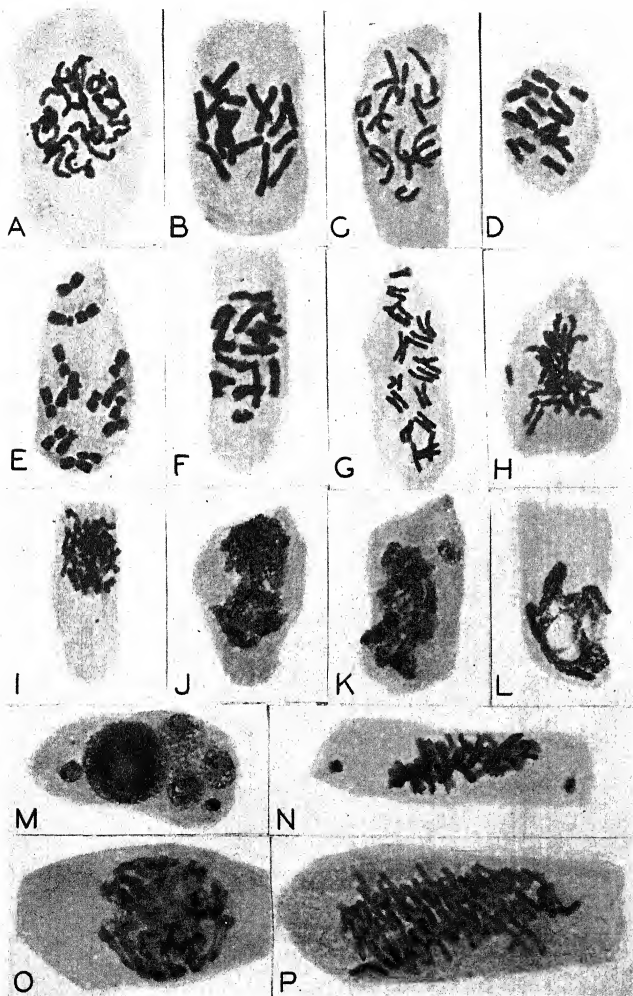
MOVEMENT OF 2,4-DICHLOROPHENOXYACETIC ACID STIMULUS AND ITS RELATION TO THE TRANSLOCATION OF ORGANIC FOOD MATERIALS IN PLANTS

JOHN W. MITCHELL[1] AND JAMES W. BROWN[2]

Introduction

When 2,4-dichlorophenoxyacetic acid or some other chemically related growth-regulating substance is applied to one part of a sensitive plant, the plant often responds in an entirely different part which may be located some distance from that to which the chemical is applied (2, 15). The effectiveness of 2,4-dichlorophenoxyacetic acid as a weed-killer is due in part to the systemic nature of its effect. It is important, therefore, in connection with its use as a herbicide (5, 7, 8, 9, 12, 14), to understand the manner in which the stimulus is translocated and the factors influencing such translocation.

The application of a relatively small amount of 2,4-dichlorophenoxyacetic acid to the stem of a succulent plant, such as tomato or bean, generally results in an increase in the rate of stem elongation (11, 16). If the compound is applied in minute amounts to one side of the stem and not to the other, then the treated side grows faster than the other and curvature of the stem results. Such curvature indicates that the treatment has resulted from the presence of a growth stimulus in at least one side of the stem—a growth stimulus in the sense that cell elongation is accelerated. A stem curvature resulting from the application of the chemical to a leaf blade, petiole, or cotyledon is an indication that a growth stimulus has been translocated from the treated region to that part of the stem in which the growth response occurs.

Using stem curvature resulting from applications of 2,4-dichlorophenoxyacetic acid as an indication of the presence of a growth stimulus, experiments were undertaken to determine the path of translocation and the effects of light and carbon dioxide on the rate of translocation of the stimulus in plants.

Experimental data

EFFECTS OF LIGHT ON TRANSPORT OF STIMULUS

EXPERIMENT I.—Preliminary experiments in collaboration with Chemical Warfare Service, Camp Detrick, Maryland, indicated that light was an important factor in connection with the response of plants to 2,4-dichlorophenoxyacetic acid.

In the present experiments, snap bean seedlings that had been grown in a shaded part of the greenhouse were selected for similarity in size and stage of development. Two plants were illuminated by means of Daylite fluorescent tubes so that the light intensity was approximately 350 foot-candles at the surface of their primary leaves. Two cameras were focused, one on each plant, in such a way that repeated photographs could be made to record growth responses that resulted from treating the plants with 2,4-dichlorophenoxyacetic acid.

One-hundredth of a milliliter of an

aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5 % by weight of Carbowax 1500 was applied as a drop to the upper surface of one primary leaf of each plant. The drop was placed at a point on the midrib approximately 5 mm. from the tip of the leaf. The initial photograph of each plant was then made, after which one plant was left exposed to the light, while the other was immediately covered with a light-proof box. Subsequent photographs of each plant were made on the original film at intervals of 4, 8, and

22 hours after treatment, the box being removed from the covered plant just long enough to make the exposure.

The treated leaves of both plants responded to the presence of the chemical, since the tip of each one curved upward, but the leaf on the plant in darkness responded more slowly than did the leaf on the illuminated plant (fig. 1). A growth stimulus was apparently translocated from the tip of the illuminated leaf along the midrib through the petiole and into the stem, where it stimulated growth along that side of the stem to which the

FIG. 1.—Growth stimulus translocated from leaf tip into stem of plant in light (A, curved); not translocated in plant in darkness (B, straight). Ten micrograms of 2,4-dichlorophenoxyacetic acid in 0.001-ml. solution of 0.5% Carbowax 1500 in water pipetted onto tip of one primary leaf of each plant. Multiple exposure photograph (repeated exposure of same plants) of each plant shows 1, position of leaves at time of treatment; 2, 22 hours later, and intervening exposures at 4 and 8 hours.

leaf was attached. In contrast, the stimulus was apparently not translocated from the leaf to the stem of the plant kept in darkness in sufficient amounts to bring about curvature of the stem. The first internode of the plant in darkness, however, increased 28.1 mm. in length in a vertical direction during the 22 hours immediately following treatment, while that of the illuminated plant increased only 15.7 mm. in length. Failure of the stem of the plant in darkness to respond could therefore not be explained on the basis of lack of growth. This experiment was repeated three times, and in each repetition the results were similar.

EXPERIMENT II.—An experiment was run to determine whether the stems of bean seedlings in a comparable stage of development were sensitive to 2,4-dichlorophenoxyacetic acid when the chemical was applied directly to one side of the stem and the plant subsequently kept in darkness. For comparison, 10 micrograms of the acid was applied, as in experiment I, to the upper surface of a primary leaf of one plant, while an equal amount was applied unilaterally to the stem of another plant. Both were then kept in complete darkness.

The stem to which the acid was applied developed a marked curvature during the 3 hours immediately following treatment. In contrast, the stem of the plant bearing the treated leaf failed to develop a curvature during a 24-hour period following treatment, at the end of which time the experiment was discontinued (fig. 2). This experiment was repeated and similar results observed.

It is concluded from the data in experiments I and II that the stems of the plants used in these experiments grew and were sensitive to 2,4-dichloro-

phenoxyacetic acid, regardless of whether the plants were illuminated or kept in complete darkness. The growth stimulus resulting from application of the acid to the leaves, however, was translocated from the leaves to the stems only in the presence of light, indicating that its movement was associated with the products of photosynthesis and the translocation of organic food materials.

EXPERIMENT III.—An experiment was run to determine whether application of

FIG. 2.—Stem curvature resulted from application of 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture to stem (1), but not to leaf (2). Both plants grown in darkness after treatment. Photographed 24 hours after treatment.

2,4-dichlorophenoxyacetic acid to a leaf relatively low in readily available carbohydrates would cause the stem of the plant to respond when the treated leaf was exposed to diffused light of low intensity. Two plants were selected for uniformity, and 0.01 ml. of an aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was applied to the upper surface (approximately 5 mm. from the tip) of one primary leaf of each plant. Both plants were illuminated by means of a Mazda reflector lamp of the spot-light type. The light intensity was

approximately 800 foot-candles at the surface of the primary leaves. The treated leaf on one of the plants was subjected to diffused light by fastening a card about 2 inches above the treated leaf so that a shadow was cast over its surface. The untreated leaf of this plant and both the treated and untreated leaves of the second plant were exposed to direct illumination.

The upper part of the stem of the fully illuminated plant bent from a vertical to a horizontal position within 6 hours after treatment, an indication that a growth stimulus had been translocated from the treated leaf to the stem. In contrast, the stem of the plant bearing the treated leaf that was shaded continued growth in a vertical position. The experiment was discontinued at the end of a 17-hour period following treatment.

The preceding experiment was repeated, but instead of applying 10 micrograms of the acid to the blade of the shaded leaf, this amount was applied as a band about 2 mm. wide around the petiole and approximately 12 mm. from the point where the petiole joined the stem. The leaf blade and petiole were shaded by means of a card as previously described, while the opposite leaf of the plant was left unshaded. As a check, the petiole of a leaf on another plant was treated with 10 micrograms of the acid in a similar manner, but instead of being shaded, the leaf was exposed to direct light from a Mazda reflector-type lamp.

A growth stimulus was translocated from the petiole of the treated illuminated leaf, as indicated by the fact that a marked curvature developed along the stem of this plant within 3 hours after treatment. The stem of the plant bearing the shaded leaf failed to bend during the same period of time (fig. 3). The card was then removed and the treated leaves

of both plants illuminated for an additional 3 hours. During this period the stem of the plant to which the previously shaded leaf was attached developed a marked curvature, and its appearance was then identical with that of the plant receiving direct illumination throughout the experiment.

EXPERIMENT IV.—An effort was made to determine the effect of 2,4-dichlorophenoxyacetic acid on the growth of seedling bean plants when the acid was applied to their leaves while growing in sunlight, as compared with the effect of similar treatment applied to the leaves of plants grown in a shaded place. Forty plants having primary leaves that were approximately two-thirds fully expanded were selected. On a clear day, 0.01 ml. of an aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was placed on the upper surface of each primary leaf. Twenty of the plants were then placed in a shaded location (250–500 foot-candles at noon on a clear day); the remaining plants were placed on an adjacent bench and exposed to sunlight of intensities that prevailed during the following 3-week period.

Stems of the plants in sunlight developed marked curvatures within a period of 2 hours following treatment. Plants in diffused light developed only slight curvatures of the leaf blades during the same period, but no response was observed in the stem. The following day the plants in direct light were curled and twisted, while those in the shade had developed slight curvatures of the treated leaves but no apparent response in the stems. One week after treatment, all the plants grown in sunlight were twisted and their terminal buds had not increased appreciably in size since treatment. Of the plants in the shade, 25%

showed moderate stem curvatures 1 week after treatment, and the terminal buds of most of them were growing vigorously. Three weeks after treatment, 70% of the plants grown in sunlight were dead as the result of treatment. The remainder had not grown noticeably following treatment. Only 30% of the growth was checked on all the plants, the majority of the plants being killed. In contrast, the stimulus was apparently not translocated from the leaves of the shaded plants in sufficient amounts to result in death of more than one-third of them or to check the growth of the remaining ones.
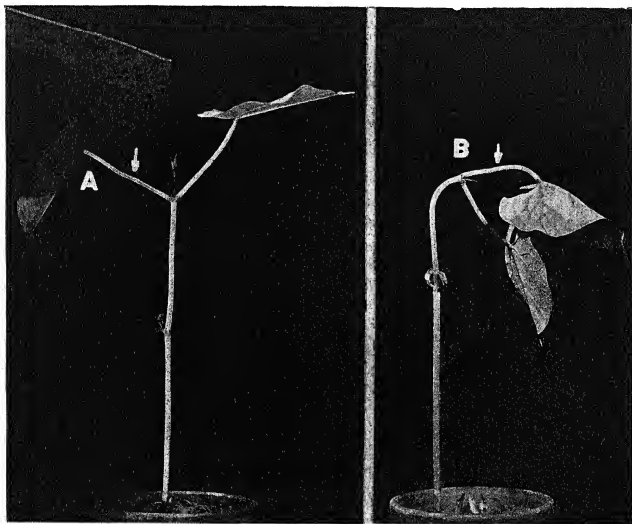

FIG. 3.—Stimulus not translocated to stem from treated shaded leaf (A, straight); translocated to stem from treated unshaded leaf (B, curved). Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to one petiole of each seedling (arrows). Plants illuminated from above. One leaf blade and treated petiole of plant A was shaded; both leaves of plant B illuminated. Photographed 3 hours after treatment.

plants grown in the shade were dead at this date; all the remaining ones were growing vigorously (fig. 4).

It was deduced from these results that a stimulus had been translocated from the leaves to the stems of plants grown in sunlight and that as a result the bud

EFFECT OF CO_2 SUPPLY ON TRANSPORT OF STIMULUS

METHODS.—Two bean seedlings were selected for uniformity, the plants having been previously grown in a greenhouse during dark, cloudy weather. Their primary leaves were nearly fully expanded

and the trifoliate leaves were beginning to unfold. As previously described, 10 micrograms of 2,4-dichlorophenoxyacetic acid was placed in solution on the upper surface of one primary leaf of each plant at the point on the midrib approximately 5 mm. from the tip of the leaf. While still attached, each treated leaf was inclosed in a cellophane envelope and the envelope sealed along its edges by means of a

a glass jet. The jet, held along the side of the petiole by means of a clamp, extended loosely through the opening for a distance of about 5 mm. into the envelope. Another air stream, from which the CO_2 had not been removed, was blown in a similar manner into the envelope inclosing the leaf of the second plant. The two air streams were adjusted to flow at approximately the same rate. A camera

FIG. 4.—Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to both primary leaves of bean seedlings generally failed to inhibit growth of bean plants grown in shade (A), but severely checked growth of those grown in sunlight (B).

tacking iron. An opening about 1 cm. in length was left unsealed through which the petiole extended. The two plants were placed under a battery of Daylite fluorescent tubes which provided a light intensity of approximately 500 footcandles at the surface of the primary leaves. A stream of air was passed through a tube of soda lime to remove the CO_2 and then directed into the opening in one of the envelopes by means of

was focused on each of the plants so that lapsed-time photographs could be taken to record growth behavior.

RESULTS.—Within 6 hours following treatment, the stem of the plant whose leaf was supplied with air containing a normal amount of CO_2 developed a marked curvature that pulled the envelope free from the stationary air jet. In contrast, the stem of the plant whose leaf was supplied with CO_2-free air failed to

develop a curvature during the same period (fig. 5). The experiment was repeated three times with similar results. Stem curvature did not develop in plants whose treated leaves were surrounded by CO_2-free air, even during a period of 17 hours following treatment.

partially unfolded at the beginning of the experiment.

Primary leaves from twenty of the plants were harvested at 8:30 A.M. for sugar analysis. In harvesting, the leaves were plunged in boiling water for a period of 2–3 seconds. The water was then

FIG. 5.—Lack of CO_2 apparently prevented translocation of stimulus from leaves of bean plants. One primary leaf of each seedling treated near tip with 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture and each sealed in cellophane envelope. CO_2-free air blown into envelope at left, and unmodified air into envelope at right. Both plants illuminated. Multiple exposure photographs taken immediately following treatment (both plants straight), and 5 hours later (plant receiving CO_2, curved).

TRANSLOCATION OF STIMULUS BY LEAVES AS RELATED TO THEIR SUGAR CONTENT

METHODS.—One hundred and forty seedling bean plants were selected for size and uniformity from a group of several hundred grown in potted soil in a greenhouse. Primary leaves of the plants were approximately one-half fully expanded and the trifoliate leaves were

shaken from them, and the blade of each primary leaf was detached from the petiole, laid on a paper tray, and dried at 80° C. in a well-ventilated oven.

At approximately the same time, one primary leaf on each of four of the plants was treated by placing a measured amount of aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid near the tip of the leaf, as

previously described. The four treated plants were placed in complete darkness at 75° F., immediately following treatment (8:30 A.M.). All the remaining plants were placed on a greenhouse bench and exposed to sunlight. Subsequent samples for sugar analysis were collected in the manner described at 9:00, 9:30, 10:00, 10:30, and 11:00 A.M. Each time a sugar sample was taken, four plants were selected at random, treated with 10 micrograms of the acid as described, and immediately placed in complete darkness to test their ability to translocate the re-
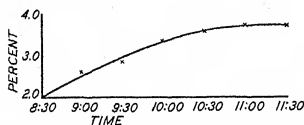

FIG. 6.—Total sugar content of leaf blades of bean seedlings at intervals during period of 3 hours' exposure to sunlight. Results expressed as percentage dry weight.

sulting stimulus. Growth responses of the plants kept in darkness were recorded during the following 48-hour period. The dried leaf samples were ground to 80-mesh size and analyzed for total sugars (10).

RESULTS.—Three hours after treatment, those plants treated at 8:30 A.M. and placed in darkness had developed very slight curvatures along the petioles, which raised the treated leaves upward; but this response was only temporary, and at the end of 6 hours the plants had returned to their original upright position. Within 6 hours after treatment, plants treated at 9:00 A.M. developed slight curvatures along the upper part of their stems, and this response was persistent. Those treated at 9:30 A.M. developed a definite and more extreme stem curvature than did the plants treated

earlier. All plants treated at 10:00 A.M. or later developed extreme stem curvature and all remained bent and distorted.

The average sugar content of leaves harvested at 8:30 was 2.1% on a dry-weight basis. It increased gradually during the first 2 hours the plants were exposed to sunlight, then leveled off at approximately 3.5% during the remainder of the experiment (fig. 6). The intensity of growth response in the stems of those plants whose leaves were treated with 2,4-dichlorophenoxyacetic acid was approximately zero at 8:30 A.M. but increased with subsequent tests until it reached a maximum in plants treated after approximately 3 hours' exposure to sunlight (fig. 7).

TRANSPORT OF STIMULUS FROM LEAVES IN DIFFERENT STAGES OF DEVELOPMENT

EXPERIMENT I.—At an early stage of development, the primary leaves of bean seedlings unfold from between the cotyledons, turn green, and increase in area very rapidly. It was thought that these rapidly growing leaves might, for a short period at least, utilize more carbohydrates than they are able to synthesize. If this were true, the flow of sugar in the petioles of these young leaves would be mainly from the cotyledons through the stem and petiole to the leaf blade. The blades of some of these young leaves were therefore treated with 2,4-dichlorophenoxyacetic acid in order to determine whether a growth stimulus would be translocated to the stems of the seedlings.

To facilitate this experiment, two potted bean seedlings were selected for uniformity when the plants were just emerging from the soil. They had been exposed to several hours of sunlight and the primary leaves were deep green in

color but still folded loosely between the cotyledons. One-hundredth milliliter of an aqueous solution containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was placed on one primary leaf of the first plant. An equal amount of the acid was placed on one cotyledon of the second seedling. Both plants were then illuminated by means of a Mazda reflector lamp of the spot-light type. The intensity of light was approximately 800 foot-candles at the level of the plants. Within 3 hours

amount of the acid had been placed grew vertically and its stem showed no evidence of curvature during the experiment. This same experiment was repeated twice with similar results. In a subsequent experiment, 50 micrograms of the acid was applied to rapidly expanding leaves of several seedlings without causing stem curvatures to develop within the following 24-hour period (fig. 8).

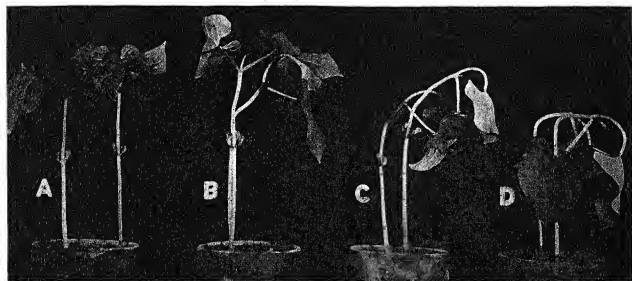EXPERIMENT II.—Further to study the rate of translocation of a growth

FIG. 7.—Response of bean seedlings to 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to tip of one primary leaf of each plant. Plants treated and immediately placed in darkness (A), and after 1 (B), 2 (C), and 3 (D) hours' exposure to sunlight.

after treatment, the stem of the plant bearing the treated cotyledon developed a curvature of approximately 90° away from the side to which the treated cotyledon was attached. By the following day the stem of this plant had developed a curvature in the opposite direction, or toward the side to which the treated cotyledon was attached, and it grew in this direction parallel to and slightly above the surface of the soil for a distance of 3–4 inches during the next 4 days, at the end of which time the experiment was discontinued. The plant bearing the primary leaf upon which an equal

stimulus through leaves in different stages of development, four bean plants of uniform size were selected. The leaves of these plants had an average area of 7 sq. cm. Four older plants having primary leaves that averaged 14 sq. cm. in area, and an equal number of still older plants with primary leaves that were fully expanded (19.5 sq. cm.), were also selected. Ten micrograms of 2,4-dichlorophenoxyacetic acid was placed on the midrib near the tip of one primary leaf of each plant in the way previously described. All the plants were then illuminated by means of fluorescent tubes.

Three hours after treatment, the youngest plants showed no evidence of stem curvature, stems of plants having medium-sized leaves showed moderate curvature, while stems of the oldest plants showed marked stem curvature. During the 24 hours immediately following treatment, leaves of the youngest plants increased in area but the stems did not develop curvature until the second day following treatment. These

Fig. 8.—Stems and very young leaves of vigorously growing bean plants sensitive to 2,4-dichlorophenoxyacetic acid but stimulus not translocated from leaves at this stage. Fifty micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture distributed as 5 drops over blade of rapidly growing leaf. Each drop contained 10 micrograms of the acid (A). One drop of same mixture containing 10 micrograms of acid applied unilaterally to stem of another seedling (B). Both plants subsequently grown in greenhouse and photographed 48 hours later.

results would indicate that the growth stimulus was not translocated from the youngest leaf blades into the stems in appreciable amounts. Partially expanded leaves were moderately effective in translocating the stimulus, while the stimulus was readily translocated from the fully expanded leaves to the stems of the plants.

PATH OF TRANSLOCATION OF STIMULUS

METHODS AND RESULTS.—An experiment was run to determine whether the growth stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid to bean plants was translocated mainly in tissues of the phloem or in the xylem. Twelve bean seedlings of uniform size were selected from a large number of plants that had been grown during cloudy weather in a greenhouse. The plants were divided into four equal groups.

One primary leaf of each plant of the first group was treated as previously described with 10 micrograms of the acid placed along the midrib near the tip of the leaf. Transport of organic food materials through the petiole of each of these treated leaves was then blocked by killing with steam the tissues in a section of the petiole. Five milliliters of an aqueous mixture containing 1000 p.p.m. of the acid and 0.5% of Carbowax 1500 was applied to the surface of the soil surrounding the roots of each of the plants in the second group. The transport of organic food materials in the stems of plants of this group was then blocked by killing the cells in a section of the stem approximately 25 mm. above the soil level. Ten micrograms of 2,4-dichlorophenoxyacetic acid was applied to one primary leaf of each plant in the third group, and 5 ml. of the acid mixture was applied to the soil around each of the plants in the fourth group, as previously described but without blocking, and these two groups were designated controls. All the plants were illuminated by means of a battery of fluorescent tubes, the light intensity at the surface of the leaves being approximately 500 foot-candles.

The stems of plants bearing treated leaves in which transport through phlo-

em and parenchyma cells of the petioles had been blocked grew vertically and showed no evidence of growth stimulation. In contrast, control plants treated in a similar manner, but with petioles intact, showed marked stem curvature (fig. 9). Further, all the plants growing in treated soil showed marked stem curvature (fig. 10).

It was concluded from these results

ever, the stimulus was apparently carried in the xylem, since it readily passed through a section of stem in which the phloem and parenchyma cells had been killed.

Effect of removing treated portion of plants

Ten micrograms of 2,4-dichlorophenoxyacetic acid was placed on one primary

Fig. 9.—Stimulus resulting from application of 2,4-dichlorophenoxyacetic acid to leaf not translocated through dead tissue in petiole. Ten micrograms of acid in aqueous Carbowax mixture applied as drop to one primary leaf of each plant. Wire splints attached to petiole of each treated leaf. Petiole of treated leaf on one plant left intact (A); segment of petiole on remaining plant killed with steam (B). Photographed 24 hours after treatment.

that the growth stimulus resulting from application of the acid to leaves was translocated mainly through the phloem or parenchyma cells, since blocking the phloem in the petiole prevented the movement of the stimulus from the leaf to the stem. When the acid was applied to the root system of the plants, how-

leaf of a bean seedling. The drop of aqueous mixture containing the acid was placed near the tip of the leaves and a narrow band of lanolin was smeared across the leaf so as to limit the liquid containing the acid to a definite area. The plant was illuminated by means of artificial light. A camera was focused on

the plant so as to record growth responses by means of lapsed-time photographs.

Marked stem curvature developed within 3 hours after treatment (fig. 11). The treated portion of the leaf was then cut off and the experiment continued for an additional period of 3 hours. At the end of this time the plant had recovered and again grew in a vertical position. This experiment was repeated and similar results observed.

In a subsequent experiment, one primary leaf of each of twelve bean seedlings was treated with 10 micrograms of 2,4-dichlorophenoxyacetic acid as described. The plants were divided into three groups of four each and another group of four plants left untreated as controls. All plants were grown in direct sunlight under prevailing greenhouse conditions. The treated areas of leaves of the first group were removed at the end of 2 hours following treatment, those of

FIG. 10.—Stimulus moved upward through dead tissue in stem of plant, resulting in curvature above killed portion. Five milliliters of aqueous mixture containing 0.1% 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 applied to soil around roots of each plant. A, stem of plant left intact; B, section of stem killed with steam. Photographed 24 hours after treatment.

leaves of the second group were removed after 4 hours, and those of the third group were removed at the end of a 6-hour period.

Stems of all treated plants developed curvatures, but this effect was not persistent in plants from which the treated areas were removed within 2 hours. A slight curvature persisted in stems of the second group, while a marked curvature of the stems persisted in the stems of the plants treated for 6 hours.

Discussion

The application of 2,4-dichlorophenoxyacetic acid to the leaves of some species of plants has resulted in the translocation of a stimulus from the treated portion of the leaves, through the petioles, and into the stems of the plants, where it has brought about apparent growth and form changes (11, 15). Application of 4-chlorophenoxyacetic acid to the stems of other plants has resulted in the translocation of a growth stimulus through the stems into the roots (2). In the present study on bean, however, it was observed that the stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid was not readily translocated from the leaves in which the sugar content was relatively low, such as those exposed to light of low intensity over extended periods of time or to CO_2-free air; nor was it readily translocated from the very young, rapidly growing leaves. Although the exact nature of the stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid is yet unknown, it is evident that its translocation from the leaves into other parts of the plant is associated with the translocation of organic food materials from the leaves into other parts of the plant. The present experiments indicate that the stimulus was continuously moved from the treated areas of leaves into the stems, under conditions favorable for the translocation of organic food materials. Although the stimulus was translocated from treated primary leaves into the stems, it was apparently not moved upward through the petioles of the opposite leaves. This observation was also made in earlier work by MITCHELL and HAM-
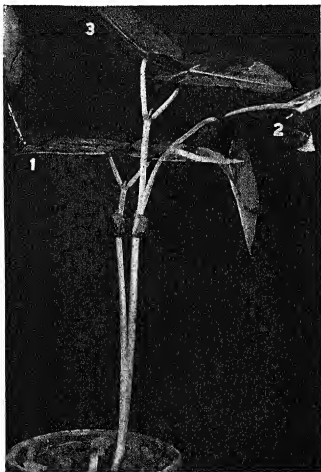
FIG. 11.—Plants recovered from effects of 2,4-dichlorophenoxyacetic acid following removal of treated area of leaf. Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied as drop at tip of leaf blade (1). Stem curvature developed during following 3-hour period (2). Treated portion of leaf then removed. At end of another 3-hour period, plant had resumed its original upright position.

NER (11, fig. 5). RABIDEAU and BURR (13) state that C_{13} likewise was not translocated from one primary leaf of a bean plant into the opposite one, although DOUTT (6) showed evidence of a vascu-

lar connection between the two primary leaves of bean plants of Black Valentine variety. BEAL (1) reported that indole-acetic acid was conducted mainly in a basipetal direction through small detached segments from the stems of bean plants.

Translocation of the stimulus from the leaves of bean plants is apparently confined to living tissues, which indicates that it travels mainly in the phloem and parenchyma cells. The translocation of certain viruses is also associated in a similar manner with the transport of organic food materials and with living cells in the plants (3, 4). When 2,4-dichlorophenoxyacetic acid was applied to the root systems of the plants in the present experiments, however, the stimulus readily passed through dead stem tissues, which indicates that with this mode of application the stimulus was carried in the transpiration stream of the xylem and independently from the translocation of organic food materials. It has been reported that carbon is not translocated through dead tissues in the stems of bean plants (13).

In using 2,4-dichlorophenoxyacetic acid as a herbicide against small annual weeds, it is doubtful whether the effectiveness of the acid is very often limited by failure of the plant to transport the stimulus. The stems and leaves of these plants can easily be covered with the spray, and transport of the stimulus for any distance within the plant is not a critical factor. Translocation of a 2,4-dichlorophenoxyacetic acid stimulus may be important, however, in connection with the eradication of deep-rooted perennials, or leafy acaulescent perennials such as dandelion and plantain, particularly when these plants are growing vigorously in shaded areas. Reduced light intensity does not favor the synthesis of carbohydrates by leaves and its subsequent translocation to other parts of the plant. On the basis of the present experiments it would appear that weeds which readily regenerate from their roots could be most effectively treated with 2,4-dichlorophenoxyacetic acid by spraying the tops during a period when organic food materials are being actively translocated from their leaves and stems to their roots.

Bean plants developed a marked response as the result of applying 2,4-dichlorophenoxyacetic acid to their leaves but recovered when the treated portions of the leaves were removed within a few hours after treatment. This would indicate that treated leaves of such plants as plantain and dandelion, or the tops of deep-rooted weeds, should not be mowed, since the effectiveness of the treatment might in this way be reduced.

Summary

1. When 2,4-dichlorophenoxyacetic acid is applied to the outer surface of some species of plants, a stimulus is translocated from the treated region to other parts, where a visible growth response occurs. The effect of light, CO_2, location of treatment, and stage of development of the plant on the rate of translocation of such a stimulus in snap-bean plants is recorded.

2. The stimulus resulting from treatment with 2,4-dichlorophenoxyacetic acid was not readily translocated from leaves of bean plants whose sugar content was relatively low, such as those exposed to extended periods of darkness or to CO_2-free air in light. The stimulus was not translocated from young rapidly growing leaves to the stems of the plants.

3. When the acid was applied to leaves of bean plants, the translocation of the

resulting stimulus was closely associated with the translocation of organic food materials.

4. Movement of the acid stimulus from leaves of bean plants apparently occurred as a continual flow under conditions favorable for carbohydrate translocation, and was confined to living cells, probably those of the phloem or parenchyma. When the acid was applied to the root system, however, the resulting stimulus was translocated through non-living cells of the stem, which indicates that it probably traveled in the transpiration stream of the xylem.

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GERMINATION OF SEEDS IN SOIL CONTAINING 2,4-DICHLORO-PHENOXYACETIC ACID

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Introduction

The use of 2,4-dichlorophenoxyacetic acid and other growth-regulating substances as herbicides (1–7) has directed attention toward the effect of these compounds on the germination of seeds and the growth of plants in soil contaminated with weeds. The germination of seeds of white clover sown just prior to a spray application of 2,4-dichlorophenoxyacetic acid to the surface of soil in field plots was suppressed; after a period of 58 days following the application there was no effect on germination of seeds of cereal grains and pasture plants (2, 5, 6). Similar results were also obtained in greenhouse experiments (5). Results of both field and greenhouse experiments indicate that the acid is apparently inactivated by or readily leached from the soil.

The present experiments were undertaken to determine the effect of 2,4-dichlorophenoxyacetic acid on the germination and emergence of seeds in soil containing known amounts of the chemical and to determine the length of time required for the acid to become inactivated in soil stored under various conditions. Another object was to study the possibility of using this compound in preventing the germination of weed seeds in soil.

Methods

Alluvial soil (2300 lb.) of a heavy clay loam type, was secured from the vicinity of Arlington, Virginia. The soil was relatively low in organic matter.

The entire batch of soil was first thoroughly mixed by hand. The acid (m.p. 141° C.) was then mixed with aliquots of it so as to make nine different concentration levels—0.1, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mg. of acid per pound of soil.

In preparing the concentration of 0.1 mg. per pound, 10 mg. of the acid was first carefully mixed with 100 gm. of dry quartz sand with the aid of a spatula. This sand-acid mixture was then thoroughly mixed by hand with 1 pound of air-dry soil. Ninety-nine pounds of soil were then weighed out and a 20-pound aliquot of it placed in a mechanical cement mixer especially designed for mixing soil. The original acid-sand-soil mixture was then added and the machine operated for 4 minutes, during which time the acid-sand-soil mixture was assumed to be evenly distributed throughout the soil, as indicated by the even distribution of grains of white sand. A second 20-pound aliquot was then added, followed by 4 minutes of mixing, and so on until the original acid-sand-soil mixture had been evenly distributed throughout the entire 99 pounds to make a total of 100 pounds of soil. This entire process was then repeated, and the two 100-pound batches of treated soil were mixed together mechanically for a period of 5 minutes.

The eight remaining soil concentrations of 2,4-dichlorophenoxyacetic acid were prepared in a similar manner, except that larger amounts of the acid were used. A final 200-pound batch of soil was mechanically mixed with 200 gm. of sand

and designated as untreated soil or control. All batches of soil were stored in wooden bins in an air-dry condition in a greenhouse. The average moisture content of the soil at the beginning of the storage period was 13.2%.

Mustard, a cultivated variety (Southern Giant Curled) of *Brassica japonica*, barley (Beardless), and morning-glory (*Impomea lacunosa* L.) seeds were used in testing the acid-soil mixtures and the untreated soil. To facilitate these tests, aliquots of each soil mixture were removed from the bins at intervals during the storage period and placed in 4-inch clay pots. Seeds of the test plants were then sown in equal numbers in soil from each of the mixtures and also in an aliquot of untreated soil. The pots containing the various soils were arranged on a greenhouse bench in the form of randomized blocks. Each plot consisted of five pots, and there were three or four replications of blocks containing all treatments. The number of seeds per pot varied from six in the case of morning glory to twelve in the case of mustard. After planting the seeds, sufficient water was added to each pot just to moisten the soil, care being taken to avoid flushing the pots when water was applied subsequently. Soil used in each test was discarded following the experiment, and new aliquots were used for each subsequent test. Results are expressed as percentage emergence, which is designated here as the percentage of plants that appeared above the soil surface calculated on the basis of the number of seeds planted.

Investigation

EXPERIMENT I: AIR-DRY SOIL.—Tests on the acid-soil mixtures using mustard were made after these mixtures had been stored in an air-dry condition for 1

month. As little as 0.1 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil (1 part of acid in $4\frac{1}{2}$ million parts of soil) reduced the number of plants that appeared above the soil by approximately 10%. Although the magnitude of reduction was not great at this concentration, it was highly significant on a statistical basis.

The effectiveness of the acid in preventing the emergence of mustard seed-
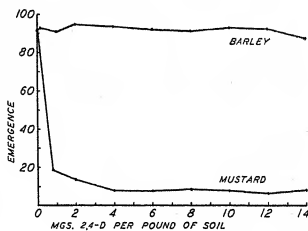
FIG. 1.—Percentage emergence of barley compared with that of mustard plants in soil mixtures containing various amounts of 2,4-dichlorophenoxyacetic acid and stored previous to planting for 1 month in air-dry condition. Counts made after complete emergence, 14 days after planting.

lings increased with the addition of larger concentrations of the acid in the soil up to 4 mg. per pound. In the last case, only 8% emergence was obtained. Greater concentrations significantly failed to reduce the percentage emergence below this level (fig. 1; table 1). This result is to be expected, since the seeds were planted approximately $\frac{1}{4}$ inch below the surface of the soil and they germinated within 3 days, during which time a few of them, no doubt, failed to come in contact with sufficient acid to prevent their emergence, even at the higher concentration levels. If the acid were added in equal amounts during the autumn to soil containing seeds under field condi-

tions, they would be exposed to the acid over a much longer period prior to germination the following spring. Under these conditions their growth would probably be more adversely affected than in the present greenhouse experiments.

Although some plants appeared above the surface of soil containing 1 mg. or more of acid per pound, their growth was greatly reduced and all but a few of them in soil containing 6 mg. or more of acid

TABLE 1

PERCENTAGE EMERGENCE OF MUSTARD SEED-LINGS IN SOIL CONTAINING 2,4-DICHLORO-PHENOXYACETIC ACID, COMPARED WITH THAT IN UNTREATED SOIL. SOIL STORED AIR-DRY. AT INTERVALS, ALIQUOTS WERE USED FOR EMERGENCE TEST AND THEN DISCARD-ED. FIGURES BASED ON FOUR REPLICATIONS OF FIVE POTS EACH, TWELVE SEEDS PER POT

2,4-DICHLOROPHENOXY-ACETIC ACID PER LB. SOIL (MG.)	SOIL STORAGE PERIOD (MONTHS)		
	1	4	6
0.	88	93	87
0.1.	80	. . . .	. . . .
1.0.	18	77	. . . .
2.0.	14	. . . .	52
4.0.	8	54	. . . .
6.0.	8	. . . .	48
8.0.	9	56	. . . .
10.0.	8	. . . .	49
12.0.	7	42	. . . .
14.0.	9	. . . .	8

per pound died within about 3 weeks after planting (fig. 2).

Subsequent tests on the acid-soil mixtures stored in a dry condition were made after 4 and 6 months of storage. The effectiveness of 2,4-dichlorophenoxyacetic acid in preventing the emergence of mustard plants decreased during this period (table 1). In a final test made on some of the batches of soil after 18 months' storage in a dry condition, the number of mustard plants that appeared above the surface of the soil 4 days after planting was markedly re-

duced. In this test, percentage emergence was 50, 40, 12, 2, and 0 in soil originally containing 0.1, 1.0, 4.0, 8.0, and 14.0 mg. of acid per pound of soil, respectively.

Tests on the effects of the acid-soil mixtures on the emergence of barley were initiated 12 days after the acid had been mixed with the soil. Subsequent tests were made at the end of 1, 6, and 18 months of storage. No significant difference was found between the number of barley plants that emerged from untreated and from treated lots of soil after complete emergence had taken place. This response was in marked contrast to the behavior of mustard seeds planted in soil containing the various amounts of acid (figs. 1, 2).

On the other hand, the early growth of the barley plants was retarded in all the acid-soil mixtures after they had been stored air-dry for a period of only 12 days (table 2). After the air-dry soil mixtures had been stored for 1 month, those containing 0.1–12.0 mg. of acid per pound did not significantly retard the early growth of barley plants. The early growth of only those plants in soil containing the highest level (14 mg. per pound) was significantly retarded, as shown by an analysis of variance of the data (table 2). Subsequent tests made 6 and 18 months after the acid was mixed with the dry soil showed no significant reduction in the early growth of barley, even in soil to which the acid had been added at the rate of 14 mg. per pound. On the basis of these tests with barley, the injurious effects of 2,4-dichlorophenoxyacetic acid in the soil mixtures decreased during the first 6 months of storage, even though the soil was kept in a relatively dry condition.

It is evident from experiments with dry soil that: (a) the presence of as little as 1 part of acid in $4\frac{1}{2}$ million parts of

soil reduced the emergence of mustard by about 10%, an amount statistically significant; (b) higher concentrations of the acid in soil reduced the emergence of mustard by about 90%, and most plants that appeared above the surface of these soil mixtures subsequently died; (c) on the basis of tests with mustard (a plant relatively sensitive to 2,4-dichlorophenoxyacetic acid) the toxic effects de-

Tests using barley plants also indicated that there was a very gradual decrease in the toxic effects of 2,4-dichlorophenoxyacetic in soil stored in an air-dry condition.

EXPERIMENT II: MOIST SOIL.—Soil of the Chester loam type (1000 lb.) was collected near Beltsville, Maryland, thoroughly mixed by hand, and divided into five batches of equal weight. Forty
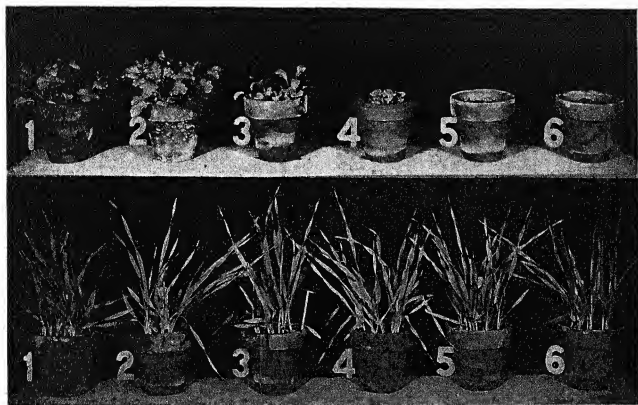

FIG. 2.—Effect of 2,4-dichlorophenoxyacetic acid in soil on emergence and early growth of mustard compared with that of barley plants: 1, untreated soil stored air-dry for 1 month prior to planting; 2, 0.1; 3, 1.0; 4, 2.0; 5, 6.0; and 6, 14 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil stored for an equal period before planting.

creased very gradually as a result of storage of the soil-acid mixtures under air-dry conditions. The total emergence of barley plants was not reduced by the presence of the acid in soil stored under dry conditions, although the rate of emergence was retarded in tests made after the acid-soil mixtures had been stored for only 12 and 30 days. After 6 months' storage, however, the highest concentrations of the acid did not significantly retard the rate of emergence.

pounds of well-rotted manure and 45.5 gm. of 5-10-5 mineral fertilizer were thoroughly mixed into each batch of soil with the aid of the mechanical mixer as previously described. One batch of soil was set aside and designated control. Into the remaining batches of soil quantities of acid were mechanically mixed as previously described, so as to result in concentrations of 0.1, 1, 6, and 12 mg. to each pound of soil. All batches of soil were then moistened evenly and stored

in wooden bins. Equal amounts of water were added with stirring to each batch at intervals during the storage period, in order to maintain the soil in a moist but crumbly condition. The germination and emergence of seeds in the various batches of soil were tested repeatedly as previously described.

The emergence of mustard plants in acid-soil mixtures rich in organic matter and stored in a moist condition was test-

FIG. 3.—Growth of barley in untreated soil (1) compared with that in soil containing 14 mg. of 2,4-dichlorophenoxyacetic acid per pound (2). Photograph taken 3 weeks after planting. Soil-acid mixture stored air-dry for 12 days before planting. Comparable test made after mixture had been stored air-dry for 6 months showed no apparent difference between early growth of plants in treated and untreated soil.

ed on the seventh, twenty-eighth, and ninetieth day after the acid had been mixed with the soil. Emergence of mustard plants in the soil mixtures containing 0.1–12.0 mg. of the acid per pound was not significantly less than that of seeds planted in untreated soil which had been stored for equal periods of time under similar conditions (table 3). In tests made after the soil mixtures had been

stored for 28 days, the emergence (calculated on actual count) of mustard plants sown in soil containing 0.1 and 1.0 mg. of the acid per pound was significantly greater by 10.2–13.7% than when sown in untreated soil. The percentage of emergence of barley plants in tests made on the same date was also significantly greater for treated soils than for untreated, the increases being 18.9, 12.0, and

TABLE 2

EARLINESS OF EMERGENCE OF BARLEY PLANTS GROWN IN SOIL CONTAINING 2,4-DICHLORO-PHENOXYACETIC ACID COMPARED WITH THAT OF PLANTS GROWN IN UNTREATED SOIL STORED IN AIR-DRY CONDITION. FIGURES REPRESENT PERCENTAGE OF PLANTS FROM 180 SEEDS THAT EMERGED DURING PERIOD OF 3–6 DAYS AFTER PLANTING

2,4-DICHLORO-PHENOXYACETIC ACID PER LB. OF SOIL (MG.)	SOIL STORAGE PERIOD		
	12 days	1 month	6 months
0............	39.8	24.5	47.0
0.1..........	29.3*	31.2	....
1.0..........	23.5*	25.3	46.7
2.0..........	30.3*	24.5	....
4.0..........	28.4*	21.5	45.7
6.0..........	28.3*	28.3	....
8.0..........	18.0*	22.5	45.0
10.0..........	·24.0*	23.3	....
12.0..........	24.8*	18.3	44.0
14.0..........	20.5*	7.5*	....

* Significantly less than emergence in untreated soil. Difference required for significance: 6.5, 7.4, and 3.2 for soil stored 12 days, 1 month, and 6 months, respectively.

18.0% in soil containing 1, 6, and 12 mg. of acid per pound of soil, respectively. After 28 days of storage in a moist condition there was no significant difference between the rate of emergence of morning-glory plants in the treated as compared with that in the untreated soils. There was, however, a slight but apparent retardation in the subsequent growth of seedling morning-glory plants in the soil mixture that originally contained 12 mg. of acid per pound of soil.

EXPERIMENT III: AIR-DRY COMPARED WITH MOIST SOIL.—Chester loam type of soil (800 lb.) was collected near Beltsville, Maryland, and thoroughly mixed by hand. It was divided into eight batches of 100 pounds each. Twenty pounds of well-rotted manure and 22.7 gm. of 5-10-5 mineral fertilizer were mixed with each 100-pound batch of soil by means of a mechanical mixer as previously described. Two of the batches of soil were set aside as controls. Sufficient 2,4-dichlorophenoxyacetic acid was then mixed individually with two other batches to make a concentration of acid equal to 5 mg. per pound of soil in each. Two other batches were made up to contain 10 mg. per pound of soil, while the remaining two were made up to contain 20 mg. The method already described for dispersing the chemical evenly throughout the soil was used. One batch of soil at each concentration level and an untreated batch were moistened and stored in wooden bins. The others were stored in a like manner but in an air-dry condition. The water content of the moist soil was kept relatively constant by the addition of small amounts of water at intervals during the storage period. Only enough water was added to keep the soil damp.

The effect of the acid-soil mixtures on the germination of seeds was determined at intervals during the storage period by planting mustard and barley seeds in aliquots taken from the various soil mixtures as previously described.

The effect of the acid-soil mixtures on the early growth of mustard and barley plants was recorded in terms of the retardation in the date of emergence and by visual observations. The effect of acid-soil mixtures on the growth and development of the plants was not recorded after the plants reached a height of 3–5

inches, since it was felt that data on the later growth and development of plants grown in such small pots would not be significant.

In experiment III, emergence tests were made simultaneously on soil mixtures containing 2,4-dichlorophenoxyacetic acid stored in a moist but crumbly condition, and on other similar soil mixtures stored in an air-dry condition. The toxic effects of the acid on germination

TABLE 3

AVERAGE PERCENTAGE EMERGENCE OF MUSTARD SEEDLINGS IN MOIST SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID AND RELATIVELY HIGH IN ORGANIC MATTER CONTENT, COMPARED WITH MOIST UNTREATED SOIL STORED UNDER SIMILAR CONDITIONS. FIGURES BASED ON FOUR REPLICATIONS OF FIVE POTS EACH, TWELVE SEEDS PER POT

2,4-DICHLOROPHENOXYACETIC ACID PER LB. SOIL (MG.)	SOIL STORAGE PERIOD (DAYS)		
	7	28	90
0	67	61	81
0.1	73	69	80
1.0	68	67	73
6.0	74	62	79
12.0	65	63	74

and the emergence of mustard seedlings decreased rapidly when the acid-soil mixtures, rich in organic matter, were stored in a moist condition at a moderate temperature (table 4). After the moist soil had been stored for 1 week, the number of plants that emerged from the batches of soil that originally contained 5, 10, and 20 mg. of the acid per pound was significantly less than the number that emerged from the untreated soil. At the end of 2 weeks of storage, however, 5 mg. of acid per pound of moist soil did not result in reduced emergence. Instead of a decrease in emergence at the 10 mg.-per-pound concentration, there was an increase of approximately 30%

(on basis of actual count) in the number of mustard plants that grew above the surface of the soil, a stimulation in growth which was statistically significant in comparison with the number of plants that emerged from the untreated soil (table 5). After 15 weeks' storage, slightly more plants emerged (10%) from batches of soil containing 10 and

TABLE 4

AVERAGE PERCENTAGE FINAL EMERGENCE OF MUSTARD PLANTS GROWN IN MOIST AND DRY SOIL RICH IN ORGANIC MATTER AND CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID, COMPARED WITH THAT IN COMPARABLE UNTREATED SOIL STORED UNDER SIMILAR CONDITIONS. EACH FIGURE BASED ON TOTAL OF 180 SEEDS. TESTS MADE AT INTERVALS DURING SOIL STORAGE PERIOD

2,4-DICHLO-ROPHENOXY-ACETIC ACID PER LB. SOIL (MG.)	SOIL STORAGE PERIOD (WEEKS)					
	Wet			Dry		
	1	2	15	1	2	15
0	67	64	68	61	67	86
5	59	66	68	31	61	79
10	36	75	75	17	48	66
20	21	53	75	6	24	49

20 mg. of the acid per pound of the soil that was stored in a moist condition than emerged from untreated soil stored under similar circumstances.

In acid-soil mixtures stored in an air-dry condition, 2,4-dichlorophenoxyacetic acid became partially inactivated. The rate of inactivation, on the basis of the germination and early growth of mustard plants, proceeded at a much slower rate in soil mixtures stored in an air-dry condition than it did in those that were kept moist (tables 4, 5).

Discussion

The extreme sensitivity of seeds, germinating seeds, and young seedlings of some kinds of plants to the presence of 2,4-dichlorophenoxyacetic acid in soils is demonstrated in the present experiments. It has been repeatedly shown that species vary in their sensitivity to this chemical when it is applied to their aerial parts. On the basis of the present data, differences in sensitivity between species are also shown when seeds are planted in soil containing this compound. These differences in response are so pronounced that concentrations of 2,4-dichlorophe-

TABLE 5

TOTAL EMERGENCE OF MUSTARD PLANTS IN MOIST AND DRY SOIL CONTAINING 2,4-DICHLORO-PHENOXYACETIC ACID AND STORED FOR 2 WEEKS, COMPARED WITH THAT OF PLANTS IN UNTREATED SOIL WITH SIMILAR MOISTURE AND STORAGE CONDITIONS. FIGURES REPRESENT TOTAL NUMBER OF SEEDLINGS THAT HAD EMERGED ON THE SPECIFIED DATE FROM 60 SEEDS PLANTED

2,4-DICHLO-ROPHENOXY-ACETIC ACID PER LB. SOIL (MG.)	MOIST SOIL										DRY SOIL									
	R₁			R₂			R₃			Av.	R₁			R₂			R₃			Av.
	1/5	1/9	1/20	1/5	1/9	1/20	1/5	1/9	1/20		1/5	1/9	1/20	1/5	1/9	1/20	1/5	1/9	1/20	
0	15	34	35	9	36	37	19	42	44	30.1	45	52	52	28	33	28	30	40	41	38.8
5	18	36	33	17	41	40	32	34	35	31.8	23	39	40	18	40	35	24	37	34	32.2*
10	28	39	41	28	45	45	40	49	49	40.4*	16	30	34	7	27	29	11	27	24	22.7*
20	11	23	26	20	40	41	19	31	30	27.2	2	16	22	2	15	13	1	8	9	9.8*

* 4.86 and 4.40 required for significant difference between average emergence, moist and dry soil, respectively.

noxyacetic acid in soil which resulted in 90% reduction in emergence of mustard plants from seeds sown in it did not inhibit the germination and growth of barley grains. In certain freshly prepared acid-soil mixtures, however, the germination and early growth of barley plants was markedly reduced. The exact nature of the decrease or elimination of the destructive or growth-depressing effects of the acid during storage of the soil mixtures is not known at present. The rate of such inactivation depends to some extent on the moisture content of the soil, being relatively slow in dry soil and surprisingly rapid in warm, moist soil having a high content of organic matter. This initial high toxicity and subsequent inactivation is of particular interest in connection with the use of 2,4-dichlorophenoxyacetic acid in the prevention of germination of weed seeds in soil, in organic matter, or in mixtures of soil and organic materials which may be incorporated into the soil, used as mulches or other types of soil amendments.

The fact that 2,4-dichlorophenoxyacetic acid was inactivated in moist soil in the absence of leaching suggests the possibility that the compound may be decomposed or rendered inactive by some types of soil organisms. It is known that some fungi and bacteria will grow readily on agar media containing as much as 0.1% 2,4-dichlorophenoxyacetic acid (8). There is also a possibility that the acid is chemically inactivated by some component of the soil.

The relatively higher percentage emergence of both mustard and barley plants in some acid-soil mixtures (6–12 mg. per pound) which had been stored for several weeks, as compared with results from untreated soil, is of special interest. These results were obtained when the soil mixtures were relatively high in organic mat-ter. Such increases in growth need not be wholly attributed to the effect of the 2,4-dichlorophenoxyacetic acid directly on the plants. The presence of the acid in the soil may have altered the rate of growth or metabolism of soil organisms, resulting in an acceleration of digestion of the organic matter in the soil and making its derivatives more readily available The entire interrelation is complex but is of great theoretical and applied significance in relation to other observations related to the growth of plants on certain soils which have been treated with mixtures of 2,4-dichlorophenoxyacetic acid for various reasons.

On the basis of the data presented here, it is apparent that the effectiveness of the acid in killing weed seeds and seedlings in soil will depend in part upon the organic and moisture content of the soil, upon the temperature to which it is subjected, and upon the soil flora and fauna present. Critical experiments involving the effects of these factors on the rate of inactivation of 2,4-dichlorophenoxyacetic acid in various types of soils must be made before this compound can be specifically recommended for killing weed seeds in areas to be used subsequently for the production of crop plants sensitive to the acid. On the basis of the relatively wide difference in sensitivity of various plants to 2,4-dichlorophenoxyacetic acid, especially cereals as compared with many kinds of dicotyledonous plants, it would appear that a suitable method of treatment of soil with the acid could be developed to effect a differential direct killing of certain kinds of seeds or young seedlings when these are present as mixtures in soils, organic manures, and the like. Or such media could be pretreated for the destruction of some or all kinds of seeds they might contain and subsequently be sown to crops

of economic importance without damage
to them.

Summary

1. 2,4-Dichlorophenoxyacetic acid was
mixed in various amounts with batches
of soil which were then stored, some air-
dry and others moist, for different peri-
ods of time. Seeds of mustard, barley,
and annual morning glory were planted
in aliquots of the various soil mixtures at
intervals during the storage periods and
the effect of the acid-soil mixtures on the
emergence of the seedlings observed.

2. One-tenth milligram of 2,4-dichlo-
rophenoxyacetic acid per pound of soil
significantly reduced the percentage of
emergence of mustard planted in the
acid-soil mixture that had been stored
air-dry for 1 month, and concentrations
of 1–14 mg. reduced its emergence by
80–90% below that of plants in untreat-
ed soil.

3. Although tests with mustard
showed that 2,4-dichlorophenoxyacetic
acid was slowly inactivated when mixed
with air-dry soil, mixtures that con-
tained 1 mg. or more per pound of soil
still reduced the emergence of the plants
by a significant amount after a storage
period of 18 months.

4. The rate of emergence of barley
plants was retarded by all concentrations
of 2,4-dichlorophenoxyacetic acid (0.1–
14.0 mg. per pound) in tests made after
the acid-soil mixtures had been stored in
an air-dry condition for 12 days. After
1 month of storage the rate of emergence
of such plants was retarded in a soil mix-
ture having 14 mg. of acid per pound,
while the lower concentrations of the acid
stored in dry soil 1 month did not signifi-
cantly affect the rate of emergence or the
final number of plants emerging. After
6 months' storage the rate of emergence
of barley plants was not adversely affect-
ed by any of the acid-soil mixtures
studied.

5. After a storage period of 1 month
in an air-dry condition, soil containing
4 mg. 2,4-dichlorophenoxyacetic acid per
pound reduced the emergence of mustard
plants by approximately 90%, but in the
case of barley the rate of emergence or
total number of seedlings that emerged
in another sample from the same batch
of soil was unaffected.

6. 2,4-Dichlorophenoxyacetic acid was
readily inactivated when mixed with
warm, moist soil. Twenty milligrams per
pound in soil relatively high in organic
matter, which had been kept warm and
moist during storage for a period of 2
weeks or longer, did not significantly re-
duce the emergence of mustard plants.

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EFFECT OF SPRAY MIXTURES CONTAINING 2,4-DICHLORO-PHENOXYACETIC ACID, UREA, AND FERMATE ON THE GROWTH OF GRASS

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Introduction

This report concerns one of a series of experiments on the selective herbicidal properties of 2,4-dichlorophenoxyacetic acid and the effects of this and related chemicals on the growth of grasses. In experiments already reported (6), attention was called to the fact that the rate of growth of established Kentucky bluegrass, fescue, and redtop was sharply reduced for a period following spray applications of 2,4-dichlorophenoxyacetic acid when the acid was applied in a concentration range that was selectively herbicidal. In appearance the grasses often do not show injury (1–5), and clipping weight measurements taken at intervals following treatment indicate that the grass plants gradually recover from the effects of the chemical (6).

In earlier studies, the control plots were hand-weeded at the beginning of the experiment, so that weed competition was not a factor and the effect of the acid on the growth of the grass could be evaluated. The experiments reported here were undertaken to study the effect of eliminating weed competition in turf through the use of 2,4-dichlorophenoxyacetic acid. In addition, efforts were made to determine whether the addition of a nitrogenous fertilizer, urea, or the organic fungicide Fermate (ferric dimethyl-dithiocarbamate) to the spray mixture would be advantageous in counteracting

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the initial retarding effects of the sprays on the growth of grasses.

Experimentation

EFFECT OF SPRAYS WITH ELIMINATION OF WEED COMPETITION

METHODS.—On March 27, 1945, plots in a weed-infested area of well-established sod were sprayed with 2,4-dichlorophenoxyacetic acid, and the growth of grasses was measured by obtaining weights of grass clippings at intervals throughout the succeeding 5-month period (May, June, July, August, and September). In this experiment the fresh clippings from the control plots were sorted by hand, and weed leaves were weighed separately from those of the grasses to facilitate measurement of the effect of the chemical as well as of weed competition on growth of the grasses.

The grasses growing in the area selected consisted of a mixed stand of Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and redtop (*Agrostis alba*). A rather dense and uniform infestation of narrow-leaved plantain (*Plantago lanceolata*) was present, with occasional plants of other species of weeds and a small amount of white clover sparsely interspersed throughout the area. On the above date the area was marked off into plots, 2 × 25 feet, which were sprayed individually with 1000 and 3000 p.p.m. 2,4-dichlorophenoxyacetic acid aqueous sprays that contained 1.0% and 2.0% Carbowax 1500, respectively. The sprays were applied

with the aid of a knapsack sprayer at a uniform rate of 5 gallons per 1000 sq. ft. Plyboard shields were held along the boundary of each plot to avoid drift of the mixtures during application. The sprays were assigned at random to the plots so as to make four blocks with an unsprayed control plot in each, a total of twelve plots.

The grass was mowed lengthwise through the center of each plot with an 18-inch lawnmower fitted with a grasscatcher. The mower cutting bar was adjusted to cut $1\frac{1}{2}$ inches above the ground. Two weeks prior to making each weight record, the entire area was mowed crosswise of the plots to provide overall uniformity in the height of the grass.

RESULTS.—During the 2-week period immediately following application of the sprays (April 10; table 1) there was a reduction (22.7%) in the growth of grass on plots treated with 3000 p.p.m. of the spray. Spray applications of the 1000 p.p.m. concentration likewise caused a reduction of 5.5% in growth of grass. These data are in accord with results previously reported (6), which showed a reduction in the growth of grass immediately following application of 2,4-dichlorophenoxyacetic acid.

The plantain plants in the treated plots were not dead by April 10, but most of their leaves were curled tightly downward and very few (less than 0.5% of the total weight of clippings) were cut off by the mower in passing over them at a cutting height of $1\frac{1}{2}$ inches. Evidence as to the effectiveness of the sprays in eradicating plantain was shown by the complete absence of this weed on May 17 and thereafter. Occasional leaves of clover and sheep sorrel were clipped from the sprayed plots in late summer, but the amounts collected were neglible.

At the end of 51 days (May 17) the grass in the sprayed plots apparently had recovered. Considerable variability in the clipping weights occurred at this time, so that the increases in growth of grass of 15.8 and 7.4% for the 1000 and 3000 p.p.m. treatments over the controls were not significant at the 5% level. However, at later clipping dates (June 26, July 20, August 14, and September 20) the increase for sprayed plots was in all instances highly significant.

The greatest increases in growth of grass, 68.9 and 80.6%, in the sprayed plots were found on July 20 following a period of weather conditions not particularly favorable for such growth. Heavy weed growth and competition with the grass in the control plots were readily observable at this time and are reflected in the small amounts of grass clippings collected from these plots. During the latter part of the experiment (record dates August 14 and September 20) seasonal weather conditions apparently were more favorable for the grass and less favorable for weed growth. As a result, the increases in grass growth owing to elimination of weeds by selective herbicidal sprays were proportionally reduced. Total rainfall (inches) during each month of the experiment was as follows: April, 3.26; May, 3.44; June, 5.13; July, 9.99; August, 1.37; September, 4.56; October, 1.46.

EFFECT OF ADDITION OF UREA AND FERMATE TO SPRAY MIXTURE

METHODS.—On August 27, 1945, an adjoining sod area composed of grasses and weed plants similar to those of the previous experiment was marked off into plots, 2×25 feet. The area had been fertilized in March with 600 lb. per acre of commercial 10–6–4 fertilizer. Aqueous spray treatments were applied

TABLE 1

EFFECT OF SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH
OF GRASS. WEIGHTS (GM.) OF GRASS CLIPPINGS OBTAINED FROM PLOTS AT INTERVALS
DURING GROWING SEASON. WEED CLIPPINGS FROM UNSPRAYED PLOTS SORTED OUT
BY HAND. SPRAYS APPLIED MARCH 27, 1945

SPRAY CONCENTRATION (P.P.M.)	REPLICATIONS				AVERAGE*	DIFFERENCE FROM CONTROLS (%)
	1	2	3	4		
April 10						
0............	298	352	253	340	310.8	....
1000............	361	222	375	217	293.8	− 5.5
3000............	250	250	219	242	240.3	−22.7
May 17						
0............	482	340	474	430	431.5	....
1000............	567	614	437	380	499.5	+15.8
3000............	434	529	387	503	463.3	+ 7.4
June 26						
0............	280	217	375	310	295.5	....
1000............	480	500	400	270	412.5	+39.6
3000............	475	530	450	420	468.8	+58.6
July 20						
0............	240	172	181	165	189.5	....
1000............	350	430	240	260	320.0	+68.9
3000............	360	385	420	205	342.2	+80.6
August 14						
0............	596	595	618	594	601.0	....
1000............	759	678	679	743	714.8	+18.9
3000............	704	789	658	876	756.8	+25.9
September 20						
0............	173	218	166	190	211.8	....
1000............	245	288	258	234	256.2	+21.0
3000............	210	290	288	240	250.1	+18.1

* Difference required for significance at 5% level: April 10, 95.3; May 17, 98.6; June 26, 101.8; July 20, 69.0;
August 14, 86.5; and September 20, 35.6.

to individual plots with a power sprayer operating at 200 lb. The sprays were applied at the rate of 5 gallons per 1000 sq. ft. The treatments consisted of: (a) unsprayed control; (b) urea in solution, at rate of 60 lb. per acre; (c) Fermate, 2 lb. per 100 gallons; (d) the ammonium salt of 2,4-dichlorophenoxyacetic acid, 1500 p.p.m.; (e) treatments c and d combined; and (f) treatments b, c, and d combined. Each treatment was assigned at random to each of six blocks, making a total of thirty-six plots.

Prior to spraying, grass on the entire area was cut to a height of $1\frac{1}{2}$ inches, and weighings of clippings were made at the end of 32 days (September 28) and 63 days (October 29) following treatment. During the first 3 weeks of this period the weather conditions were rather hot and dry. The clippings were collected in the same manner as previously described, those from plots that did not receive 2,4-dichlorophenoxyacetic acid being sorted and the weeds and grass weighed separately.

RESULTS.—The sod area selected was responsive to the fertilizing effects of urea. The growth of both grass and weeds was significantly stimulated throughout the experiment at both dates of clipping by the spray treatment with urea alone (table 2).

Use of the ammonium salt of 2,4-dichlorophenoxyacetic acid alone caused a reduction in growth of grass during the first month, but by the end of 2 months the grass clippings from plots of this treatment were 40.4% greater than the untreated controls. Much of this increase in rate of growth may have been due to the elimination of weed competition, since approximately 99% of the weeds were killed in all plots that received the salt of 2,4-dichlorophenoxyacetic acid, either alone or in combination

with other compounds. Urea at the rate used apparently did not interfere with the selective herbicidal effects when added to sprays containing 1500 p.p.m. of the ammonium salt of the acid. Plots that received a combination spray containing the acid salt and urea not only were free of weeds but the grass was darker green in color and produced greater leaf growth than did the unsprayed plots (table 2). The initial depressing effects of the acid salt on grass growth apparently were completely eliminated by the addition of urea to the spray solution. The greening effects of urea in this combination were evident within 3–4 days after application and continued throughout the experiment well into the cool weather of late October and early November. The growth of grass in the control plots, as well as in others (treated with Fermate) that did not receive urea alone or in combination, appeared to be more adversely affected by cold weather conditions in late October than was that in the fertilized plots. As a result, a very high increase, 131.7%, in weight of clippings was obtained from these plots when compared with the controls on October 29 (table 2).

No attempt was made to measure the fungicidal value of the Fermate treatments. It is of interest, however, that this compound when used alone or in combination with urea and the acid salt at the rate of 2 lb. per 100 gallons did not significantly affect the growth of grass or weeds. The herbicidal effects of the 2,4-dichlorophenoxyacetic acid apparently were not affected by the addition of Fermate, since the plots that received the combination spray containing 2,4-dichlorophenoxyacetic acid, urea, and Fermate were just as free of weeds as the other plots receiving comparable

amounts of the herbicide alone. Likewise, the fertilizing effect of urea on the growth of grass was not significantly affected when it was applied in spray mixtures.

Effect of varying amounts of urea in spray mixture

Methods.—On September 26, an adjacent area of sod similar to that used in the previous experiments was marked off into plots, 2 × 25 feet, to determine the effects of adding various amounts of urea to the acid spray.

Urea was applied at rates equivalent to 0, 60, 90, and 120 lb. per acre in spray solutions containing 1500 p.p.m. of the ammonium salt of 2,4-dichlorophenoxyacetic acid. Each of the four spray treatments, together with an unsprayed control, was assigned at random to plots in each of six blocks, making a total of thirty individual plots. All sprays were applied with a power sprayer at the rate of 5 gallons per 1000 sq. ft., and weights of the grass clippings were obtained as in the previous experiments. The grass leaves clipped from the different plots

TABLE 2

Growth of grass affected by elimination of weed competition through use of ammonium salt of 2,4-dichlorophenoxyacetic acid as spray, and effectiveness of this spray when urea and Fermate were added. Figures represent average weight of clippings obtained from six plots per treatment. Sprays applied August 27, 1945

Treatment*	Clipping weights (gm.)				Grass weight compared with control (%)
	Total	Weeds	Weeds (%)	Grass	
			September 28		
Control, unsprayed	191.7	48.8	25.5	142.8	....
Urea	225.0	60.7	27.0	164.3	+ 15.1
Fermate	193.3	57.0	29.5	136.3	− 4.5
Salt†	128.3	0.0	0.0	128.3	− 10.2
Salt plus urea	205.0	0.0	0.0	205.0	+ 43.6
Salt plus urea plus Fermate	200.0	0.0	0.0	200.0	+ 40.1
Difference required for significance at 5% level	33.2	....	....	20.8	....
			October 29		
Control, unsprayed	113.3	42.1	37.2	71.2	....
Urea	141.7	47.9	33.8	93.8	+ 31.7
Fermate	123.3	45.3	36.7	78.0	+ 9.6
Salt	100.0	0.0	0.0	100.0	+ 40.4
Salt plus urea	165.0	0.0	0.0	165.0	+131.7
Salt plus urea plus Fermate	165.0	0.0	0.0	165.0	+131.7
Difference required for significance at 5% level	18.0	....	....	14.6	....

* Amounts of the various ingredients used in spray mixtures were: urea (technical grade) sufficient to apply at rate of 60 lb. per acre; Fermate 2 lb. per 100 gallons; and ammonium salt of 2,4-dichlorophenoxyacetic acid at 1500 p.p.m. applied at 5 gallons per 1000 sq. ft.

† Of 2,4-dichlorophenoxyacetic acid.

were sorted out and weighed for comparison with weights of the weed clippings in each sample.

RESULTS.—As in the earlier experiment, urea applied at the rate of 60 lb. per acre in solution together with the ammonium salt of 2,4-dichlorophenoxyacetic acid did not cause apparent burning or other type of injury to the grass. At the rate of 90 lb. per acre, however, a moderate amount of tip burn was observed on the grass in all plots, while at

the initial burning effect of the strong urea concentration used.

Fresh-clipping weight measurements were obtained on all plots at the end of 42 days (November 7). As shown in table 3, no significant difference was obtained at this time in grass production as a result of urea application at rates equivalent to 60, 90, or 120 lb. per acre. After a period of 42 days the grass had apparently fully recovered from the initial foliage burn caused by application

TABLE 3

EFFECT OF AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID AND VARYING AMOUNTS OF UREA FERTILIZER ON GROWTH OF GRASS. FIGURES REPRESENT AVERAGE WEIGHT OF CLIPPINGS FROM SIX PLOTS PER TREATMENT. SPRAYS APPLIED SEPTEMBER 26 AND CLIPPINGS MADE NOVEMBER 7, 1945

TREATMENT*	CLIPPING WEIGHTS (GM.)				GRASS WEIGHT COMPARED WITH CONTROL (%)
	Total	Weeds	Weeds (%)	Grass	
Control	231.7	54.9	23.7	176.8	....
Salt	153.3	0.0	0.0	153.3	− 13.3
Salt plus 60 lb. per acre urea	233.3	0.0	0.0	233.3	+ 32.0
Salt plus 90 lb. per acre urea	238.3	0.0	0.0	238.3	+ 34.8
Salt plus 120 lb. per acre urea	246.7	0.0	0.0	246.7	+ 39.5
Difference required for significance at 5% level	30.3	....	....	30.3	....

* Ammonium salt of 2,4-dichlorophenoxyacetic acid used in all spray mixtures at 1500 p.p.m. concentration, and spray applied at rate of 5 gallons per 1000 sq. ft. Urea added in amounts sufficient to apply at indicated rates per acre.

120 lb. the grass foliage was severely damaged. The plots of this treatment were quite brown in appearance for a period of 10–14 days following application, after which time they recovered and again became green.

Narrow-leaved plantain was killed equally well (approximately 99%) in all the sprayed plots, irrespective of the amount of urea that had been added to the spray mixture. There was some indication that the plantain in those plots that received the highest rate of urea application together with the acid salt died somewhat more quickly. This behavior may have been associated with

of 90 or 120 lb. per acre of urea in spray mixture containing the herbicidal salt.

Discussion

In experiments reported previously (4, 5) it was found that certain grasses, such as the bents (*Agrostis* spp.), are quite sensitive to spray applications of 2,4-dichlorophenoxyacetic acid at concentrations that will kill dandelion and narrow-leaved plantain. Other grasses, such as Kentucky bluegrass, red fescue, and redtop, appeared to be more resistant to the sprays. Recently (6) it was found that even these more resistant grasses may be affected by the sprays,

in that they produced less top growth following treatment, usually without the appearance of leaf discolorations or other apparent symptoms that might be associated with spray injury. The reduction in leaf growth has been of short duration, and after a month or so the plants produce new leaf growth at about the same rate as the unsprayed grass.

In the experiments reported here a reduction in the growth of established grasses was also noted following spray application of 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid. In some instances the amount of reduction in growth was somewhat greater than that caused by weed competition on the plots used, but the grass soon recovered, and, as might be expected without the competing effects of weeds, grew at a much faster rate throughout the summer than did grass in the weedy control plots.

The addition of urea to the spray mixture greatly aided in bringing about an early recovery from the growth-retarding effects of the ammonium salt of 2,4-dichlorophenoxyacetic acid. The dry mixture of the ammonium salt of the acid and urea is readily soluble in water and makes a clear spray solution. From the standpoint of lawn and pasture-improvement practices, mixtures of this type appear to be useful in eradicating certain weeds and fertilizing the grass in the same operation. It is possible that other nitrogenous fertilizing agents may be more adaptable for use in selective herbicidal mixtures of this type. Under the conditions of these experiments, which were conducted on sod heavily infested with weeds and on soil relatively low in fertility, a marked stimulatory effect was obtained from urea applied at the rate of 60 lb. per acre. No burn-

ing or other injurious effects were noted from spray applications of urea at this rate, even with sprays applied under hot, dry weather conditions which are usually associated with burning injury from nitrogenous fertilizer applications to grass foliage. Applications of herbicidal spray mixtures containing urea in concentrations sufficient to apply 90 and 120 lb. per acre caused moderate to severe burning of the grass when applied under rather cool, moist growing conditions.

Tests of dry applications of 2,4-dichlorophenoxyacetic acid in combination with a complete fertilizer mixture are in progress. The preliminary results with dry applications of this type seem promising as a convenient method in the absence of spraying equipment. The herbicidal properties of the acid seem to be unaffected when applied in this manner, but much experimentation may be necessary to determine a safe and effective mixture for practical use.

Summary

1. The growth of established grasses—Kentucky bluegrass, red fescue, and redtop—growing in mixed stand with a rather heavy weed infestation of narrow-leaved plantain was measured by weighing clippings periodically during the summer following spring application of 2,4-dichlorophenoxyacetic acid spray mixtures to eradicate the weeds. The effect on growth of grass from adding urea as a fertilizer and Fermate as a fungicide to the herbicidal sprays containing the ammonium salt of the acid was also studied. Comparisons were made with weedy control plots from which the clippings of weeds and grass were sorted and weighed separately.

2. Immediately following application of the sprays (1000 p.p.m. of the acid

or 1500 p.p.m. of the ammonium salt) the rate of growth of grass was significantly reduced for a short time, 10 days to 2 weeks. The reduction in most instances was greater than that caused by the competition of a rather dense stand of weeds. However, the grass soon recovered from the spray treatment and, without the competing effect of the weeds, produced 15–80% more leaf growth on the sprayed than on the weedy unsprayed plots.

3. Application in late August of a spray mixture composed of 1500 p.p.m. of the ammonium salt of the acid and urea sufficient to apply 60 lb. per acre caused the grass to become much greener in color within a few days. The urea apparently had no adverse effect on the selective herbicidal properties of the acid salt, so that, as a result of weed elimination and fertilization, the grass in the sprayed plots produced 40–131% more growth in the 2-month period following treatment than did that in the unsprayed plots.

4. Under conditions of the experiments no burning or other injury to the grass was obtained with the herbicidal sprays to which urea equivalent to an application of 60 lb. per acre was added. However, when the urea content was increased to the equivalent of an application of either 90 or 120 lb. per acre, the grass was severely injured but recovered by the end of 1 month following treatment.

5. Although no attempt was made to measure the fungicidal properties of Fermate when applied in spray mixture with 1500 p.p.m. of the ammonium salt of the acid, it is of interest that Fermate at the rate of 2 lb. per 100 gallons in the mixture had no apparent effect on the herbicidal action of the spray.

BUREAU OF PLANT INDUSTRY, SOILS
AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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METHODS FOR STUDYING THE MAIZE EAR

One of the difficulties experienced in most morphological studies of the maize ear is that of properly dissecting or sectioning the cob. This is especially true of mature cobs, which are usually very hard. Mechanical methods, such as trimming the chaff from the cob and sandpapering, have been the usual procedure in the preparation of mature cobs. When cobs are collected young they may be sectioned or cleared, but even then the vascular system is so complex that a detailed study still may be difficult.

For study of the vascular system, the writer has developed a retting process involving the use of cellulose-digesting microörganisms, by which excellent material has been obtained. By this method, all the tissues of the cob except the vascular bundles are digested and may be washed off, leaving the entire vascular system intact and in good condition (figs. 1, 2). It is helpful to bag the young ears which are to be used as specimens to prevent their becoming pollinated. The cobs then develop to approximately normal size but only their vascular bundles become lignified, the rest of the tissue remaining parenchymatous and hence more readily digested.

For study of the woody skeleton, cobs may be prepared in any desired quantity simply by covering them with 72% commercial-strength sulphuric acid for about 12 hours and then washing off the dissolved material (fig. 3). Specimens treated in this way are useful in a study of the external morphology of the cob, and the method is an improvement over the older one of trimming and sandpapering.

An alternative reagent for softening the cob is 40–41% commercial-strength hydrochloric acid. This permits dividing the cob into segments, which are believed by the writer to be morphological units.

The preceding methods have been in use since 1939, in a study of the morphology of the maize ear. The main objective in the study has been to determine whether the fusion of simpler spikes has been a factor in the development of the polystichous character of the ear. On account of the exigencies of war, a full report of the results has not been completed and may not be available for some time. It can be stated, however, that the results of a morphological comparison of the maize ear with maize tassels

and with spikes of other grasses, such as *Sorghum halepense*, *Setaria italica*, *Pennisetum glaucum*, and *Chloris berroi*, fail to support the theory that fusion of simpler spikes has been a factor in the development of the ear.—R. G. REEVES, *Texas Agricultural Experiment Station, College Station, Texas.*
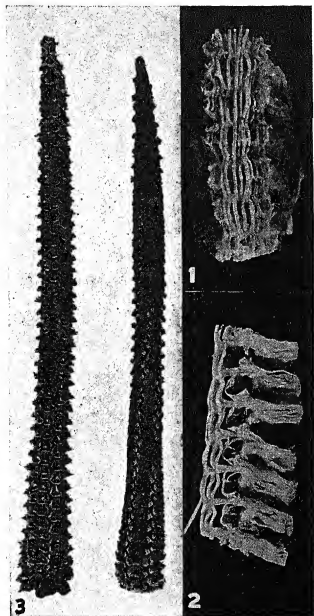
FIGS. 1–3.—Maize cobs after preparation for study: Fig. 1, interior view of portion of cob after digestion of parenchymatous tissues by action of microörganisms. Fig. 2, tangential view of portion of cob after similar treatment. Fig. 3, cobs treated with sulphuric acid, for study of external morphological characters.
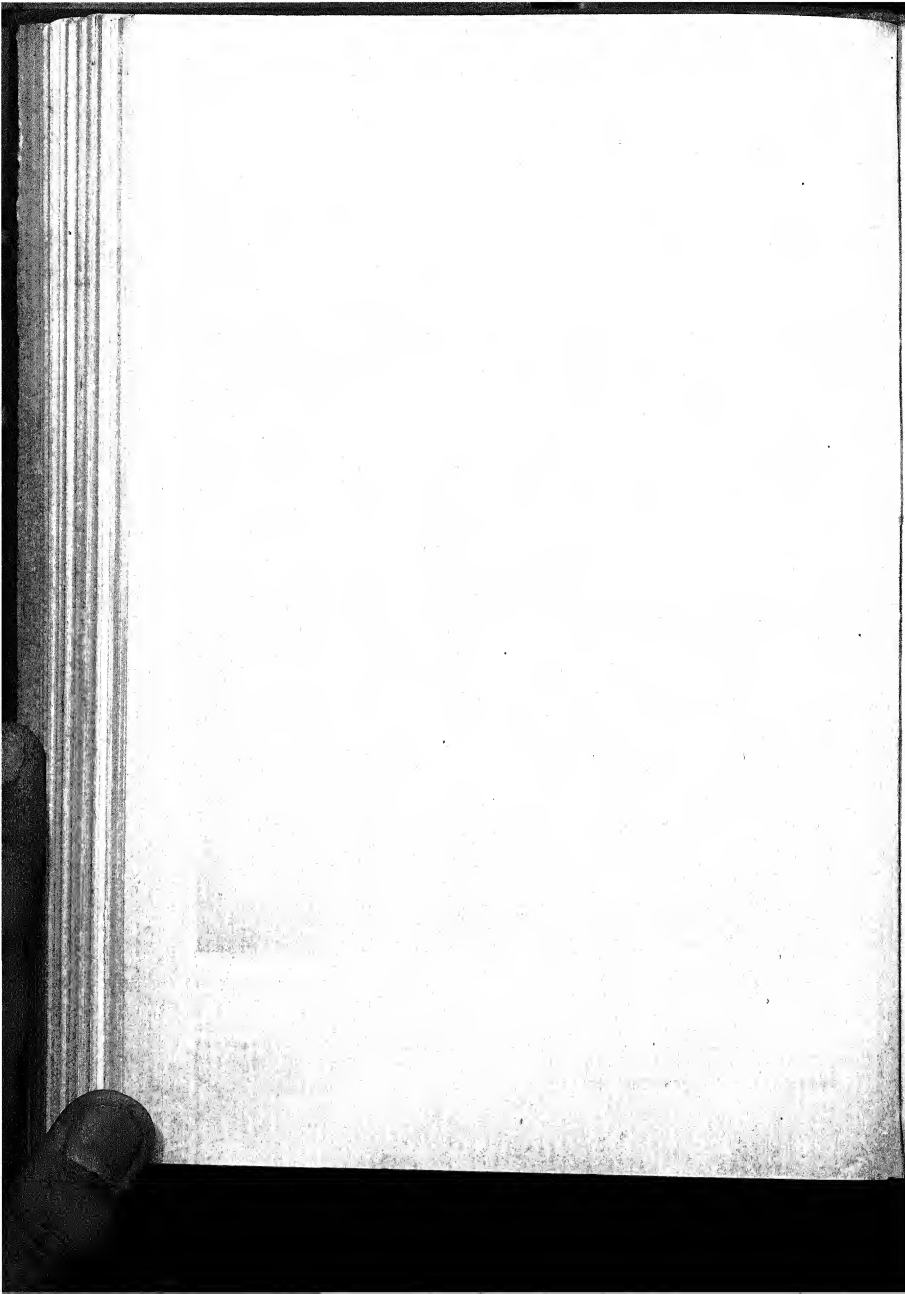
SOME EFFECTS OF SEASON, HABITAT, AND CLIPPING ON THE CHEMICAL COMPOSITION OF ANDROPOGON FURCATUS AND STIPA SPARTEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 572

ROBERT J. WEAVER

Introduction

The chemical composition of forage plants as influenced by season, habitat, and grazing has been studied by many investigators. Such studies supply information on the comparative nutritive value of plants at various stages of development, under varying environmental conditions, and under grazing. In investigations concerned with the improvement and maintenance of the forage cover, a knowledge of the nutritive value of the plants is just as important as their palatability, yield, and reaction to grazing. The chemical composition of tops of forage plants frequently grazed may be closely associated with the relatively small amounts of roots and tops usually produced by these plants.

There has been little investigation of the chemical composition of many of the grasses of the true prairie, especially in eastern Nebraska. Big bluestem (*Andropogon furcatus* Muhl.) and needle grass (*Stipa spartea* Trin.) were selected for this work, as they are dominants in the prairie, are valuable pasture grasses, and are found as chief constituents of the best native hay in eastern Nebraska. The purpose of this study was to compare the seasonal trend of the chemical constituents of these two species in two habitats and to note the effects of clipping on the composition of native and seedling plants.

Review of literature

GORDON and SAMPSON (8) and WATKINS (20) have extensively reviewed the literature concerning the chemical composition of range plants. Many investigators have found that in range plants there is a decline in the percentage of crude protein and minerals and an increase in the percentage of crude fiber from the earliest appearance of leaf blades to maturity (4, 8, 13).

The storage of sugars and starch in certain grasses was found to be inversely related to the growth rate, but hemicellulose showed a rather continued percentage increase throughout the growth cycle of the plants (14, 15, 17).

STODDART (19) concluded that season had a far greater influence upon chemical composition of *Symphoricarpos rotundifolius* than did either site or soil.

Soil type affects the chemical composition of plants in part through its effect on availability of nutrients and water (4, 5, 7). The soil type may also influence the species composition of the flora and thereby indirectly the chemical composition of the forage.

BROWN (3) used thermo-regulated growth chambers in his investigations and showed that temperature has a marked effect on chemical composition of grasses.

Forage that regenerates after grazing or clipping has been found to be very suc-

culent, with high percentages of crude protein and low percentages of crude fiber (11, 18).

Materials

Big bluestem, a sod-former, is the most important native tall grass in eastern Nebraska. Here this grass, although deeply rooted, is typically an indicator of moist but well-aerated lowland, but it also occurs in limited amounts and small size on the dry uplands. It is of southern derivation and normally resumes growth at Lincoln about April 15. Its vegetative growth is normally complete by September, after which seed is usually produced in abundance.

Needle grass is found characteristically on dry uplands and, if elsewhere, on lighter types of soil. It produces bunches usually 2–4 inches in diameter, and the root system varies usually from 3 to 5 feet in depth. It is of northern extraction and usually renews growth in eastern Nebraska early in March and flowers and produces seeds in late May or early June. It does not grow vigorously thereafter until the cooler weather of early fall but continues growth until winter.

Two stations were selected in virgin prairie about 3 miles northwest of Lincoln, Nebraska. One station was on moderately low, gently sloping land, and the other area was about $\frac{1}{4}$ mile distant on a hill-crest. The soil at the lowland station was Wabash silt loam; that at the upland station, approximately 30 feet higher, was Carrington clay loam. Topsoil on the upland was about 2 feet in depth and on the lowland about 3 feet deep. In the lowland there was an abundance of big bluestem and a small area where needle grass was dominant; the upland had large amounts of needle grass but only small bunches of big bluestem, scattered sparingly throughout.

Samples were taken throughout the growing season from the native grass at both stations, from areas on the lowland, which were repeatedly clipped at a height of 2 inches to simulate grazing, and from seedlings at both stations. The seedlings were grown in square-meter areas. In order to insure prompt, uniform germination and vigorous growth, the sod had been loosened to a depth of 2 inches, the coarser roots and rhizomes removed, and the soil well firmed, watered, and mulched after the seed had been planted.

Conditions for growth

The growing season of 1941 was characterized at Lincoln by a late spring, a wet April, moderate rainfall in May and June, and severe drought during July and August. Precipitation for April, May, and June was 3.12, 2.47, and 3.41 inches, respectively, or $+0.59$, -1.61, and -0.91 inches compared with the normal. The normal rainfall for July and August is 3.85 and 3.57 inches, but only 0.59 and 0.80 inches, respectively, fell during these months.

The mean monthly temperatures, April–August inclusive, are $54°.2$, $68°.4$, $72°.6$, $78°.8$, and $78°.5$ F., respectively. Temperatures were all above normal during 1941, excess temperatures being $+2°.7$, $+6°.7$, $+1°.2$, $+2°.3$, and $+4°.1$ for the several months, respectively.

Evaporation from an open tank operated by the Weather Bureau at Lincoln was 7.1 inches in both May and June, 10.9 inches in July, and 10.2 inches in August.

Water content of soil, based on the total amount of moisture minus the hygroscopic coefficient at the various depths at each sampling, is shown in table 1. Several years of drought had preceded 1941; and, on March 31, soil on the upland was moist to a depth of only

29–39 inches and on the lower area to a depth of 49–57 inches.

On the upland, water was available for growth until late in June, after which very little occurred at any depth. Rainfall of 1 inch on June 27 was the last shower in excess of 0.2 inch until August 23, when 0.49 inch fell. Available water content at the lowland station was greater at all times and at all depths than on growth cycle and seeded abundantly. On the upland, water was applied only occasionally, and the big bluestem failed to produce flower stalks. Needle grass, however, produced seed in June. The showers, even in May and June, were poorly distributed for growth of seedlings. Therefore, the seedlings were watered as required to make possible adequate growth. An understanding of the devel-

TABLE 1

WATER CONTENT OF SOIL IN EXCESS OF THE HYGROSCOPIC COEFFICIENT
AT LINCOLN, NEBRASKA, DURING 1941

Depth in feet	Hygroscopic coefficient	March 31	May 10	May 24	June 20	July 11	July 24	Aug. 7	Aug. 31
				Upland Station					
0–0.5	8.4	18.8	15.3	14.0	4.1	−1.2	−2.7	−0.9	−1.8
0.5–1	9.6	12.3	11.3	8.3	5.1	−0.3	−1.3	−1.1	0.6
1–2	11.0	9.8	10.8	12.1	6.0	0.6	0.0	−1.1	−0.5
2–3	10.0	4.2	8.0	8.6	5.5	0.3	1.8	−0.9	0.6
3–4	7.5	4.2	....	7.9	3.9	1.7	3.7	0.9	3.6
				Lowland Station					
0–0.5	9.4	28.2	24.5	21.1	10.6	1.0	3.2	1.7	0.8
0.5–1	10.6	18.0	17.8	16.5	11.8	1.2	0.2	1.2	0.7
1–2	12.0	10.2	15.0	13.2	9.9	2.1	1.4	0.5	0.2
2–3	12.4	8.7	13.5	12.4	10.0	4.9	6.3	1.0	0.3
3–4	12.3	7.5	....	10.1	9.2	5.1	4.8	2.7	1.1
4–5	12.4	4.6	....	6.2	....	....	0.0	3.6	2.5

the upland. It, too, decreased sharply late in June, but water remained available, although often in small amounts, until August 31. These samplings for soil-moisture determinations were made near, but not in, the areas reserved for collecting plant materials. During the dry weather the area in the lowland from which the samples were taken for chemical analysis was supplied with enough water by sprinkling so that the grasses were able to continue growth. Those on the low ground completed their regular

opment of the grass at each station at the times of sampling is essential to an interpretation of the chemical composition.

Growth of the grass

BIG BLUESTEM

Development of big bluestem during the period of growth is shown by the data in table 2. The number of leaves was less on upland plants than on lowland after June 20 because of the extreme drought on the upland. There were green leaves on the watered lowland plants at

all times, but upland plants began to turn yellow or brown by July 10, and the leaf-tips dried despite watering. Actual decrease in general level of foliage of upland plants was also a drought effect; by August 7 the ends of the leaves had died back 2–5 inches and some whole shoots had died.[1] The plants were about one-third dried and had a dull-green color. Height of plants on lowland after July 10 was that of flower stalks; the general foliage level reached a maximum of about 26 inches. Plants on the upland produced no

the summer, but grass from the first clipping (May 13) was discarded, since it was the same as that of other lowland plants collected at this time. Material for the first analysis under this treatment was secured on June 5, when there were three or four leaf blades per shoot, projecting above the 2-inch-high clipping level, and the general height of the blades was 13 inches. By June 20 the plants had made an excellent recovery; most stems had two leaf blades above the clipping level, with a general height of

TABLE 2

GENERAL FOLIAGE HEIGHTS, NUMBER OF LEAVES, AND STEM DIAMETERS OF BIG BLUE-STEM ON LOWLAND AND ON UPLAND AT TIMES OF COLLECTION OF SAMPLES

DATE	LOWLAND			UPLAND		
	General foliage height (inches)	Average no. of leaves per culm	Range of stem diameter (mm.)	General foliage height (inches)	Average no. of leaves per culm	Range of stem diameter (mm.)
May 13..........	7	3	....	6	3	....
June 5..........	18	5	3–4	10	5	2–3
June 20..........	20	5	2–5	16	5	2–3
July 10..........	22	6	3–6	14	5	2–3
Aug. 7..........	32	6	3–6	14	5	2–3
Sept. 1..........	40	6	3–6	11	5	2–3

flower stalks, and by September 1 more than half of their leaves and stems were dead, and only a few of the green leaves were turgid. On August 7 and September 1 in the lowland, the flower stalks, each with five small leaves, were collected separately from the unelongated shoots in the same sods.

CLIPPED BLUESTEM ON THE LOWLAND

Certain patches of big bluestem on the lowland were clipped six times during

[1] All height measurements reported in this paper, unless otherwise specified, are the general heights of the foliage or flower stalks in their natural condition; leaves were never straightened out in order to obtain a measurement.

10 inches. At the clipping on July 10, the foliage height was about 12 inches, and there were usually four leaves more than 2 inches high on each culm. Since some terminal growing-points did not renew growth after the earlier clippings, the stand of grass was thinned but growing vigorously. At the fifth clipping on August 7, the sturdy stems of the still thinner stand usually had five leaves which had an average height of 10 inches. On September 1, much growth of basal tillers had occurred, and there were four broad leaves per stem and an average height of 7 inches. This grass rapidly regenerates after close cutting or grazing (21).

BLUESTEM SEEDLINGS

Seed was planted on April 26 at both stations. By June 20, when the plants were first clipped, the average foliage height on the lowland was 4.5 inches. There was an average of twenty-five seedlings per square decimeter, and the plants were in the sixth-leaf stage. Many seedlings had one or two tillers, but the latter were too short to be included in the samples, which were clipped at a height of 1.5 inches. On the upland the average plant height was an inch less than for lowland plants, and there were only fifteen plants per square decimeter. The number of leaves and degree of tillering, however, was about the same as for plants on the lowland. On July 24 there was a thick stand of much-tillered plants on the lowland. They had been watered weekly and were thriving. The seedlings had about six leaves and were once more 4.5 inches tall. Those on the upland were only 3.5 inches high, and some plants were much dwarfed, despite similar weekly watering. A third clipping was secured on September 1 from the seedlings on the lowland. The seedlings were then vigorously growing; their average height was 6 inches, and nearly all had either five or six leaves each.

NEEDLE GRASS

Samples of *S. spartea* were collected seven times, April 23–September 1, inclusive. Foliage height was approximately the same at both stations at each sampling. On April 23, the general level of foliage was 5.5 inches, and the plants were in the third-leaf stage. By May 15 the general level of foliage was 13 inches, and the stems had an average of five leaves each. Inflorescences were present on some stems, but they were still inclosed in the leaf-sheaths. On June 5, flower stalks, which were harvested separately, were 36–48 inches tall when held erect. Each had three short leaves above the 2-inch cutting height; these were left on the stalks. The seeds were in the dough stage. The general level of foliage was 18 inches high, and each unelongated culm had three or four long, tapering leaves. No further increase in general level of foliage occurred. On June 20 the fruits had all been shed, and the flower stalks, which were dead and dry at least throughout their upper half, were not included in this or subsequent samples. On July 11, plants on the lowland were green and thriving; on the upland the leaves were rolled tightly because of drought. At the sampling on August 7, one-third of the leaves on lowland plants were dry or nearly dry, a usual midsummer phenomenon, and on the upland nearly one-half of the foliage was bleached white and dried. At the last sampling—September 1—the late-summer, half-dried condition prevailed on the lowland; on the upland many bunches were entirely dry, but others were one-third green.

CLIPPED NEEDLE GRASS

By May 13, twenty days after the first clipping, the plants had made much recovery. The general level of foliage was 11 inches, and there were a few flower stalks in an early stage of development. The general level of foliage was again 11 inches on June 5. Each stem usually had two narrow leaves projecting above the clipping level. Since the plants were suffering from too frequent removal of the foliage, the third sample was not taken until July 11. Each stem now had three leaves previously unclipped, with a general foliage level of 14 inches. Some bunches contained very few stems, while others were one-third filled. By August 7, little regeneration of the clipped grass

had occurred. Only ten to twenty-five stems scattered throughout the large bunches renewed growth. The general height of foliage was about 7 inches, and each culm had an average of three to five leaves each. On September 1 the general level of foliage was 6 inches, and there were three leaves or parts of leaves per stem.

SEEDLING NEEDLE GRASS

The needle grass, planted April 26, grew more rapidly in the warmer and drier upland soil, where it had attained a general level of foliage of 7 inches when clipped on June 20. Seedlings were then in the third- or fourth-leaf stage. Most plants had one or two tillers, 1.5–2.5 inches in height. The stand was open, only about 8 plants occurring per square decimeter. When the needle grass on the lowland was clipped on July 3, it had attained approximately the same size and density of stand as that described on the upland. Needle grass on the upland regenerated to such a small degree that it was not clipped again. The grass on the lowland was harvested on September 1, when the plants had a foliage level of 5 inches and an average of three leaves per culm.

Methods

COLLECTION OF SAMPLES

Each composite sample for chemical analysis consisted of plants taken in at least five different places in the local experimental area. Further samples were never taken from the same place, thus assuring the natural growth and maturing of the tissues included in each sample.

In the clipping experiments, parts of many plants were included, as an area of about 1 square meter was clipped on each collecting date. The seedlings likewise were all clipped at each of the several times of collecting, whether or not all material was used for the chemical analysis.

Samples were taken between 10:00 and 11:00 A.M. and in a definite sequence. They included the entire aerial portion of the plant, unless otherwise specified. The grass was placed in paper bags and taken at once to the laboratory, where it was then killed by being subjected to a temperature of approximately 100° C. It was placed on a 2-mm. mesh screen, and heat was applied from below by an electric heater with a large reflector. This arrangement permitted constant movement of large amounts of hot dry air. After 5–8 minutes the temperature was decreased to 70° C. for 15 minutes, and the final drying was in an electric oven at that temperature.

CHEMICAL ANALYSIS

When completely dry, the grass was finely ground and placed in tightly stoppered bottles. Duplicate samples were used for the determinations, and the analytical methods used were those described by LOOMIS and SHULL (12). The reduced-iron method adapted by PUCHER was used for determination of total soluble nitrogen.

Results

BIG BLUESTEM

It is emphasized that all analytical data in this investigation are expressed as percentage values and not as absolute amounts.

On each sampling date throughout the season, established plants on the lowland had the highest percentage of total sugars, clipped plants on the lowland the lowest, and upland plants had an intermediate percentage (table 3). Seedlings

on the upland contained about the same percentage of sugar as established upland plants, but the percentage sugar content of lowland seedlings was small.

Established plants on the upland contained the highest percentage of starch

of seedling plants in the two habitats was about equal.

The percentage sugar-starch content of established lowland grass was less than that on the upland early in the season, but it was greater on the lowland after the latter part of June, when drought be-

TABLE 3

PARTIAL CHEMICAL ANALYSIS OF ABOVE-GROUND PARTS OF BIG BLUESTEM AT SIX STAGES OF DEVELOPMENT, EXPRESSED AS PERCENTAGES OF OVEN-DRY WEIGHT

Plants	Date collected	Total sugars	Starch	Total sugars and starch	Hemi-cellulose	Soluble nitrogen	"True" protein	Total ash
Upland.............	May 13	1.7	6.8	8.5	13.8	0.30	12.5	8.5
	June 5	2.9	6.4	9.3	18.5	.17	7.7	5.3
	June 20	1.4	6.5	7.9	17.6	.16	6.1	5.5
	July 10	3.4	3.9	7.3	21.6	.12	4.8	5.2
	Aug. 7	1.8	3.7	5.5	21.4	.10	3.2	4.5
	Sept. 1	1.0	3.8	4.8	20.6	.09	2.6	5.1
Lowland............	May 13	1.9	4.6	6.5	13.2	.41	15.0	9.5
	June 5	3.2	4.1	7.3	19.5	.14	7.8	6.4
	June 20	1.9	5.8	7.7	20.3	.13	5.6	6.2
	July 10	4.5	4.1	8.6	21.6	.11	4.2	5.9
	Aug. 7	3.1	3.4	6.5	22.8	.09	3.2	5.4
	Aug. 7*	4.0	3.9	7.9	23.5	.09	2.4	4.3
	Aug. 7†	1.9	2.6	4.5	21.8	.10	4.5	7.1
	Sept. 1	2.0	4.7	6.7	23.8	.08	2.5	5.7
	Sept. 1*	2.1	4.8	6.9	24.2	.07	2.2	4.7
	Sept. 1†	1.8	4.5	6.3	23.0	.08	3.1	7.2
Lowland, clipped....	May 13	1.9	4.6	6.5	13.2	.41	15.0	9.5
	June 5	2.3	3.8	6.1	15.9	.16	9.5	8.0
	June 20	0.8	3.9	4.7	13.7	.27	11.6	9.7
	July 10	1.6	2.0	3.6	18.9	.16	8.3	7.7
	Aug. 7	1.6	1.8	3.4	18.7	.14	7.3	7.2
	Sept. 1	0.9	4.0	4.9	18.5	.14	7.1	7.6
Upland seedlings....	June 20	1.6	4.9	6.5	12.0	.16	10.1	7.1
	July 24	3.0	3.8	6.8	15.5	.18	7.2	6.0
Lowland seedlings...	June 20	0.9	4.5	5.4	12.7	.19	8.3	8.4
	July 24	2.0	3.7	5.7	18.6	.16	7.2	7.7
	Sept. 1	1.5	3.2	4.7	15.5	0.13	7.1	8.2

* Analysis of stems and flowers.
† Analysis of leaves.

(about 6.5 per cent) at the first three sampling dates (table 3). Clipped plants on the lowland contained the lowest percentage of starch except at the September 1 collection, when they approximately equaled the upland plants in percentage starch content. The starch content

came severe on the upland (fig. 1). Upland seedlings had a higher percentage content of this fraction than did those growing on the lowland. The lowland clipped plants had a gradually decreasing sugar-starch fraction during most of the season.

The percentage of hemicellulose increased generally during the growth cycle of the plants (fig. 1). Established plants on the lowland usually contained a higher percentage of hemicellulose than the corresponding upland plants. The percentage content of this fraction in seedlings at the time of sampling was less than in established plants growing near by. The clippings from established grass at the lowland station contained a smaller percentage of hemicellulose than did the unclipped plants.

On May 13, established lowland plants contained more total soluble nitrogen than did corresponding upland plants; but, after June 5, upland plants had a higher percentage of their weight in this fraction, although after June 20 the difference was probably not significant (fig. 1). Seedling plants had, in general, a higher percentage of soluble nitrogen than did the established plants. Clipped plants on the lowland, with one exception, had a higher percentage of soluble nitrogen than did unclipped plants.

The graph showing percentage changes of "true" protein is, in general, similar to the graph for total soluble nitrogen (fig. 1).

Established plants had a relatively high percentage of ash at the first sampling (fig. 1). Lowland plants had consistently about 0.5–1.0 per cent higher total ash content than did upland plants. Seedlings had a higher percentage ash content than the corresponding established plants did, and established plants on the lowland, which were clipped throughout the season, maintained a high percentage level of total ash.

The chemical composition of flower stalks and leaves at the last two sampling dates is shown in table 3. On August 7 each inflorescence was about one-half exserted, and by September 1 they were partly to fully expanded. On August 7 the flower stalks contained almost twice the percentage of sugar and starch as did the leaves, but by September 1 the percentages were approximately equal. There was slightly more hemicellulose on a percentage basis in stalks than in the leaves. On August 7 the ratio of percentage of "true" protein in flower stalks to percentage in the leaves was about 1:1.9, but by September 1 this ratio had been reduced to 1:1.4. Leaves contained about 1.6 times as much total ash as did the flower stalks on a percentage basis.

NEEDLE GRASS

The percentage content of total sugars was relatively high early in the season (table 4), and established plants on the upland usually had the highest percentage content of total sugars. These data indicate that the percentages of starch and of sugar plus starch decreased as the season advanced and that no one treatment had the highest percentages of these compounds at all samplings (fig. 2).

Established plants on the upland contained a higher percentage of hemicellulose than did the corresponding lowland plants (fig. 2). Clippings from established and seedling grass usually contained smaller percentages of hemicellulose than did unclipped plants.

Graphs showing percentage changes of total soluble nitrogen and "true" protein in the grass are, in general, similar (fig. 2). The percentage content of these constituents decreased with the advancing season, and all clipped plants had a higher percentage of their weight in these fractions than did unclipped plants.

Established plants on the lowland maintained a higher percentage ash content than did either established plants on the upland or clipped established plants on the lowland (fig. 2). Clipped seedlings
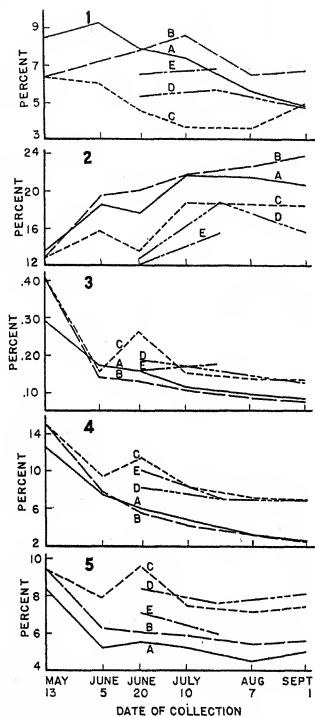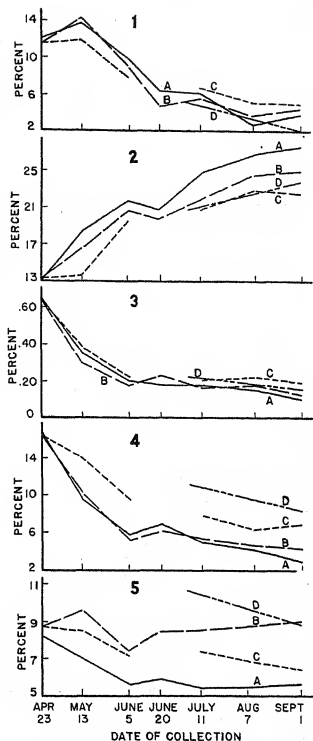
FIG. 1.—Total percentages (dry-weight basis) of (1) sugars and starch, (2) hemicelluloses, (3) soluble nitrogen, (4) "true" protein, and (5) ash in big bluestem at several stages of growth, clipped and not clipped. *A*, established plants on the upland; *B*, same on the lowland; *C*, same clipped plants on the lowland; *D*, clipped lowland seedlings; and *E*, clipped upland seedlings.

FIG. 2.—Total percentages (dry-weight basis) of (1) sugars and starch, (2) hemicelluloses, (3) soluble nitrogen, (4) "true" protein, and (5) ash in needle grass at several stages of growth, clipped and not clipped. *A*, established plants on the upland; *B*, same on the lowland; *C*, same clipped plants on the lowland; and *D*, clipped lowland seedlings.

usually had the highest percentage ash content.

The chemical composition of flower stalks and leaves of plants on the upland and on the lowland at the sampling on June 5 is shown in table 4. Flower stalks plants shortly after the early leaf stage, the further slight decrease as the season advanced, and the gradual increase of the hemicellulose fraction throughout the growth cycle are in agreement with the results of other investigators (4, 8).

TABLE 4

PARTIAL CHEMICAL ANALYSIS OF ABOVE-GROUND PARTS OF NEEDLE GRASS AT SEVEN STAGES OF
DEVELOPMENT, EXPRESSED AS PERCENTAGES OF OVEN-DRY WEIGHT

Plants	Date collected	Total sugars	Starch	Total sugars and starch	Hemi-cellulose	Soluble nitrogen	"True" protein	Total ash
Upland.............	April 23	6.3	5.7	12.0	13.1	0.65	16.8	8.3
	May 13	7.5	6.1	13.6	18.3	.35	9.6	7.0
	June 5	4.9	4.9	9.8	21.5	.20	5.7	5.6
	June 5*	5.4	5.6	11.0	21.2	.18	4.4	5.4
	June 5†	3.6	2.8	6.4	21.9	.24	8.8	6.0
	June 20	2.4	4.0	6.4	20.8	.19	6.9	5.9
	July 11	3.7	2.5	6.2	24.6	.18	5.1	5.4
	Aug. 7	0.8	1.8	2.6	26.7	.15	4.4	5.5
	Sept. 1	0.7	3.1	3.8	27.5	.10	2.9	5.6
Lowland............	April 23	6.1	5.5	11.6	13.2	.64	16.6	8.8
	May 13	7.5	6.7	14.2	16.5	.30	10.1	9.6
	June 5	4.3	4.7	9.0	20.6	.18	5.3	7.3
	June 5*	4.5	5.0	9.5	20.5	.16	4.5	6.8
	June 5†	3.5	3.8	7.3	20.8	.22	7.7	8.8
	June 20	1.3	3.3	4.6	19.6	.23	6.2	8.5
	July 11	2.1	3.1	5.2	21.8	.17	5.5	8.5
	Aug. 7	0.5	3.5	4.0	24.5	.16	4.8	8.8
	Sept. 1	0.6	3.8	4.4	24.9	.12	4.4	9.0
Lowland, clipped....	April 23	6.1	5.5	11.6	13.2	.64	16.6	8.8
	May 13	6.5	5.3	11.8	13.7	.37	13.9	8.5
	June 5	3.3	4.4	7.7	19.6	.21	9.6	7.2
	July 11	2.5	4.0	6.5	20.9	.20	7.9	7.3
	Aug. 7	1.7	3.3	5.0	22.8	.22	6.4	6.9
	Sept. 1	0.7	4.0	4.7	22.6	.18	7.0	6.4
Upland seedlings....	June 20	0.9	7.8	8.7	16.2	.25	13.0	9.3
Lowland seedlings...	July 3	1.3	3.9	5.2	20.9	.22	11.3	10.7
	Sept. 1	0.6	1.4	2.0	23.8	0.15	8.5	8.9

* Analysis of stems and flowers.   † Analysis of leaves.

had a higher percentage content of sugar-starch but lower percentages of hemi-cellulose, soluble nitrogen, "true" protein, and total ash than did the leaves.

Discussion

The rapid percentage decrease of crude protein in established, unclipped

Early in the season unclipped established big bluestem on the upland usually had a higher percentage content of sugar plus starch than did big bluestem on the lowland. After June 20, however, when drought became severe on the upland, the situation was reversed. When soil moisture became depleted on the upland,

the tips of the leaves of big bluestem began to die back and dry up, resulting in decreased photosynthetic activity. The degree of development of established unclipped needle grass on the upland and the lowland was quite similar throughout the season of growth. On June 11, however, leaves of needle grass on the upland were rolled tightly because of drought, and, later, more drying of foliage occurred on the upland than on the lowland. This may partially account for the lower sugar-starch percentage in needle grass on the upland at the last two times of sampling.

It has been shown that part of the change in composition of forage with advancing maturity is the result of leaching of soluble constituents (9). The sugar fraction could be readily leached from the dried grass leaves. Although precipitation was abnormally low during the latter part of the growing season, the rains on June 27 and August 23 of 1.00 and 0.49 inches, respectively, may have caused considerable leaching. Since much drying-out of leaves occurred in the unclipped established plants on the upland and less drying occurred on the lowland, more leaching would be expected from the former plants.

It was noted that early in the season when the percentage of starch in big bluestem was high, the percentage of sugar was low, but that, as the season progressed, the situation was reversed. Perhaps a partial explanation for this is that these fractions are closely interrelated. Investigations with frequent samplings, however, are necessary for proof that these are seasonal changes and not those of daily or short duration.

In reviewing the literature, EATON (6) noted that opinions are still in conflict as to whether acid-hydrolyzable carbohydrates (hemicellulose) are used in metabolism or whether they are permanent structural constituents of the cell wall. McCARTY (14, 15), in experiments with the wild oat (*Avena fatua*) and mountain brome (*Bromus carinatus*), found indications that the acid-hydrolyzable hemicellulose is largely employed as a structural material even by clipped plants. The percentage of hemicellulose in the unclipped big bluestem and needle grass generally increased during the advancing season, which suggests that this fraction is not used, at least in large amounts, as a reserve food. The percentage of hemicellulose in established upland big bluestem decreased slightly during the interval from June 5 to June 20. Big bluestem on the upland grew rapidly during this period, growing 6 inches in average foliage height. This rapid growth may have resulted in the addition of much succulent tissue, with a lower percentage of hemicellulose. The usually greater hemicellulose percentage in big bluestem on the lowland than on the upland may be due to the more stemmy nature of the former plants. Stems of big bluestem contain a slightly larger proportion of hemicellulose than do the leaves.

In established unclipped plants the proportion of available carbohydrates to available nitrogenous compounds progressively increased with the stage of development of the plant. These data are in agreement with the findings of KRAUS and KRAYBILL (10), who showed that when plants were vigorously vegetative but unfruitful, the proportion of available carbohydrates to nitrogen was relatively low, but in plants which were vigorous and also reproductive the relative proportion of these was intermediate.

The clippings from seedlings clipped several times during the growing season were in a succulent condition. This was reflected in the high percentages of sol-

uble nitrogen, "true" protein, and low percentage of hemicellulose. Foliage which grows after clipping or grazing is approximately as succulent as is that in the spring.

Clippings from the established plants which were periodically clipped on the lowland were also succulent and showed high percentages of soluble nitrogen and "true" protein and a low percentage of hemicellulose at the times of sampling. The rapid decrease in the percentage of the sugar-starch fraction in the clipped grasses during their development probably resulted from the rapid conversion of the products of photosynthesis into structural materials. McCARTY (14) reported that in the wild oat there was low food (sugar and starch) concentration in the herbage regenerated after cutting. He showed in mountain brome in California that the annual herbage growth and yield are products of carbohydrates manufactured currently in the herbage (15). The carbohydrates in the roots and stem bases of mountain brome were at a minimum shortly after snow disappeared in spring.

The results from clipped big bluestem and needle grass indicate that the chemical composition varies during the season, even though the herbage is maintained in a succulent condition by repeated clipping. ARCHIBOLD and NELSON (1) have stated: "Grass kept in the vegetative stage by grazing may be quite different in chemical composition in midsummer from what it was in the spring. The seasonal factors, rainfall, temperature, sunshine, and length of day, exert their influence irrespective of the stage of growth of the plant."

Since the total soluble nitrogen, "true" protein, and total ash percentages were higher in grasses remaining in the leaf stage than in plants that had developed

flower stalks, it is probable that the former plants are more nutritious forage for animals. CLARKE and TISDALE (4) found that, in junegrass (*Koeleria cristata*), common speargrass (*S. comata*), and grama grass (*Bouteloua gracilis*), leafage was generally richer in crude protein, total ash, calcium, and phosphorus, and lower in percentage of crude fiber than the culms.

An explanation of the causes for the difference in chemical composition of established plants on the upland and the lowland is difficult, because possible differences in several factors of the environment—water content of soil, humidity, light, temperature, soil solutes, and soil air—may have directly affected the plants. The chemical composition of plants is closely associated with their stage of growth. In this experiment the stages of growth of big bluestem varied greatly between plants on the upland and on the lowland; only on the lowland did flowering occur. The differences in degree of development of needle grass in the two habitats was slight, but more drying of foliage occurred in plants on the upland. Since the supply of available water was probably the most critically controlling environmental factor in determining the degree of development and drying of the plants, varying water content between upland and lowland was perhaps the primary factor associated with the chemical differences between the plants.

The variation in height and density of stand of big bluestem on the upland, as contrasted with its growth on the lowland, may have been of importance in the chemical differences. In the taller and denser stand on the lowland the lower leaves would be shaded, with corresponding limitations on their rate of photosynthesis. This might bring about variation

in the entire chemical composition of the plant.

The scattered plants of big bluestem on the upland might be genetically different from plants on the lowland. Big bluestem grows normally in low moist soil, and the plants which persisted on the dry hill-crests might be a drought-resistant strain. The scattered bunches of needle grass on the lowland might, on the other hand, be a strain adapted to a moist environment. If this were true, the same species in the two habitats might have differing chemical composition even if grown under identical conditions. The use of identical clones in each area would eliminate this possibility for error.

It is generally agreed that frequent clipping of tops of plants usually reduces the yield both of tops and of roots. In eastern Nebraska, BISWELL and WEAVER (2) found that the dry weight of tops from sods of big bluestem clipped seven times during the season of growth was only 14.5 per cent of that of unclipped plants and that the dry weight of roots from clipped plants was only 5.3 per cent of that of controls. The low percentage content of the sugar-starch fraction usually present in the clipped big bluestem and needle grass was probably associated with a decreased production of tops and roots.

These data may be of significance in estimating the value of forage plants growing in different habitats. For example, if the chemical composition of big bluestem or needle grass growing on the upland and lowland is known, one should be able to estimate their relative forage values when the analytical data are used in conjunction with the yields and palatability of the grasses in the two habitats. Digestion trials should be used in conjunction with analytical data, as it has been shown that chemical analysis when used alone may sometimes be misleading. Forages of similar composition may have different digestibilities and thus be of unequal value to the animal (16).

A knowledge of chemical composition of clippings from cut forage is important in comparing seasonal value of the clippings with that of plants allowed to grow to maturity. A study of chemical composition would be a valuable adjunct to all experiments in which the reaction of forage to clipping or grazing is studied.

Summary

1. The seasonal trend of certain chemical constituents of established big bluestem (*A. furcatus*) and needle grass (*S. spartea*) growing on lowland and upland habitats in eastern Nebraska was studied. The effects of periodic clipping on the composition of established and seedling plants were also investigated.

2. The growing season was characterized by a late spring; sufficient rainfall in April, May, and June; and severe drought in July and August. Mean monthly temperatures were all above normal. After late June little soil moisture available for growth occurred on the upland. On the lowland, water remained available, at least in small amounts, throughout the investigation.

3. Established big bluestem plants on the lowland became taller than those on the upland. The latter showed severe drought effects early in July, and flowering occurred only in the lowland plants. The degree of development of needle grass in the two habitats was similar, although more drying of foliage occurred in plants on the upland. During the flowering stage, grasses with flower stalks and grasses remaining in the leaf stage were separately collected.

Established grasses on the lowland regenerated after five cuttings. Seeds plant-

ed on the upland and lowland produced seedlings, which were clipped from one to three times.

4. The sugar plus starch percentage was greater in big bluestem on the upland than on the lowland early in the season, but later the situation was reversed. The percentage decreased in big bluestem on the upland when the leaves began to dry up. This drying may have reduced photosynthesis and may have permitted the soluble sugars to be leached from the leaves.

The hemicellulose percentage in big bluestem was usually higher in established plants on the lowland, perhaps because of their more stemmy nature. Needle grass on the upland, however, had a higher percentage of hemicellulose than lowland plants. Indications were that hemicellulose is not used as a reserve food.

5. Varying available water content between the upland and the lowland was probably the primary factor in causing the established unclipped plants in the two habitats to be chemically different, since the supply of available water was probably the most critically controlling factor in determining the degree of development and drying of the plants.

6. Clippings from established plants which were periodically clipped were succulent, and their percentages of soluble nitrogen and "true" protein were high, but their percentage content of hemicellulose was low. Clippings from seedlings showed generally similar results.

7. The soluble nitrogen, "true" protein, and total ash percentages were higher in grass remaining in the leaf stage than in plants with flower stalks, but the sugar-starch percentage was greater in the culms.

8. This investigation may be of value in comparing the forage value of big bluestem and needle grass in different habitats and under grazing.

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MODE, SITE, AND TIME OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA IN LINUM USITATISSIMUM L.[1]

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GEORGE K. K. LINK AND VIRGINIA EGGERS

Introduction

In the course of a study by EGGERS (6, 7) of the role of carbohydrate and nitrate-nitrogen nutrition in hypocotyledonary bud formation and development in the Bison variety of *Linum usitatissimum* L., tests were begun in 1936 to investigate the effects of auxins upon the development of hypocotyledonary bud primordia into buds and shoots. The results of those studies will be communicated in a later paper. During the early phases of those studies we found it necessary to reinvestigate the problems of mode, site, and time of initiation of these bud primordia in both nondecapitated and decapitated hypocotyls of the flax

plant. The results of the latter studies are reported in this paper.

The literature bearing on this topic is not very extensive. BURNS and HEDDEN (4) in 1906 reported results of their studies of the conditions which affect the appearance of hypocotyledonary buds in several plants, including *L. usitatissimum*. TAMMES (10) corroborated their findings as to the occurrence of such buds in decapitated flax hypocotyls, and ADAMS (1), apparently unaware of these reports, announced the discovery of such buds for decapitated hypocotyls of flax. BEALS (3) included the formation of hypocotyledonary buds in flax in her study of the histogenesis of regenerated organs. NEUBERT (8) made extensive studies of the regenerative activities of several species of flax, including *L. usitatissimum*. CROOKS (5) in the course of a histological study of the seedling of flax also investigated its regenerative activities and made a detailed study of the histogenesis

of its hypocotyledonary buds. A monograph dealing with the development of subcotyledonary shoots and their significance in the growth forms of plants was published by RAUH (9). He supplemented CROOK's findings with results derived from investigation of the histogenesis of hypocotyledonary buds in *L. usitatissimum* L. and *L. tenuifolium* L.

Materials and methods

After a series of preliminary studies, seed of the Bison variety of *L. usitatissimum* was planted in May, 1939, in soil and in sand in pots and between moist filter paper in Petri dishes. The seedlings emerged from the soil and sand on the 4th day. On the 5th day some seedlings were decapitated immediately below the cotyledons, the others were left uncut as controls. Samples of the uncut hypocotyls of the soil and sand plantings were collected every day for 15 days, and of the filter paper set for 3 days. In another set, samples were taken on the 36th day. The first samples of the decapitated hypocotyls were collected 2 days after decapitation (7-day-old plants) and every second day thereafter through the 10th day after decapitation. Samples also were taken 10 days after decapitation of 36-day-old plants.

The specimens taken were fixed in Crook's modification of Navashin's solution. As modified, this consists of solution A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. water), mixed in equal volumes just before using. Butyl alcohol was used for clearing. The material was imbedded in paraffin. At the beginning it was sectioned, some transversely, some longitudinally; but later all specimens were sectioned only longitudinally, 10 μ

thick. The sections were stained with Flemming's triple stain.

The sections were studied critically for evidence of bud primordia and for later stages of bud development. The sites of such activity were noted, and counts, in terms of occurrence of these sites in the lower and the upper halves of the hypocotyl, were made.

In addition, the gross behavior of the nondecapitated and the decapitated hypocotyls was noted for correlation with the results of the histogenetic studies.

Observations and findings

a) GROSS BEHAVIOR OF THE HYPOCOTYL

The hypocotyledonary axis of the 1–2-day-old seedling is characterized by a bend which turns the root downward. This bend promptly takes the shape of an inverted U and then, as it straightens, pulls the seed coats and cotyledons out of the substrate. It is the first part of the seedling to emerge from the substrate. In our May plantings straightening was completed by the close of the 4th day. The rate of these events depends upon internal factors, such as the age of the seed, and upon external factors, such as temperature, moisture, and illumination.

The bend and straightening result from successive cell elongation on opposite sides of the hypocotyledonary axis. The cells on the prospectively convex side elongate first; this causes the bend and arch. Then the cells of the concave side elongate; this causes the straightening. After this has been accomplished, a phase of zonal elongation sets in. In soil-and sand-grown seedlings, this phase of zonal elongation begins at the base of the aerial part of the axis, as reported by CROOKS (5), and then proceeds progressively upward along the hypocotyl until the zone immediately below the

cotyledons is reached. While the straightening phase usually is completed in 4–5 days, the zonal-elongation phase may require 5–15 days more for completion, but duration of this phase, too, depends upon internal and external factors.

If a decapitation cut is made in the region of zonal elongation or above it, the decapitated stump keeps on elongating, the amount of elongation depending upon the amount of actual and potential elongation zones left by the cut. For example, if hypocotyls are severed on the day of emergence of the bend, at the top of the bend while it is only 1–2 mm. above the soil, the stumps straighten, swell, become greener, and increase two to five times in length during the next 5 days. After that, elongation ceases. Such stumps develop an abundance of buds. The later and the higher up the cut is made, the less the increase in length of the stump.

b) PHELLOGENIC ACTIVITY OF THE HYPOCOTYL

Generally, by the 30th to 40th days, the nondecapitated hypocotyl begins to lose its deep-green color, and its surface begins to crack and to become scaly. In the meantime, slight but general tangential stretching in part accommodates the epidermal and other cortical cells to the diameter increase of the hypocotyl. In addition, some accommodation results from radial divisions and subsequent cell enlargement, especially in the hypodermis and deeper cortical cells (fig. 7). Tangential divisions begin to appear in the epidermal cells after the 30th day. Several of these divisions may take place within the confines of the original epidermal wall. Later, tangential division proceeds to the hypodermis, the deeper cortical cells, and the pericycle. After the first tangential division,

the derivatives may experience radial division (fig. 7). The walls of the phellem are not heavily suberized. A phelloderm is lacking. Cracking and sloughing begin in the lowest zone of the hypocotyl and proceed upward. In 90-day-old material, sloughing had extended into the pericycle.

The young decapitated hypocotyl becomes greener than the nondecapitated. It usually swells immediately after decapitation, especially at the upper end, largely because of increase in size of intercellular spaces. In time, the epidermis, deeper cortex, and pericycle successively become phellogenic, as in the nondecapitated hypocotyl.

c) MODE OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA

BURNS and HEDDEN (4) reported that the hypocotyledonary buds of *L. usitatissimum* are of epidermal origin. BEALS (3) and CROOKS (5) have verified this report. At the onset of bud-primordium initiation, the epidermis consists of cells which are longitudinally elongated. Some of these cells are two to three times as long as they are wide, many of them are much longer, and a few are shorter. Many of the epidermal cells are more or less pointed at one or both ends. When mature, their outer wall is cutinized. At this stage the guard and accessory cells are fully differentiated. The mature epidermal cell possesses a large central vacuole. Neither the cytoplasm lining its wall nor its nucleus is very prominent. Occasionally one of these cells bears one or more cytoplasmic bridges across the vacuole.

There is no grossly detectable increase in size of the epidermal cell immediately preceding the first division leading to bud-primordium initiation (figs. 1, 2), nor is there marked increase of the cyto-

plasmic contents of the cell about to divide or of the daughter cells. Neither is the position of the nucleus in a cytoplasmic strand transversely across the central vacuole a diagnostic sign of bud-primordium initiation. In fact, the cells which are about to engage in, or just formation, the cells involved become rich in cytoplasm. Neither BURNS and HEDDEN (4) nor CROOKS (5) made mention of this. Even after several additional divisions within the confines of the old cell wall, the new cells are not characterized by marked replacement of the central

FIGS. 1–3.—Fig. 1, tangential section of an 11-day-old nondecapitated hypocotyl, showing epidermal cell with transverse divisions which initiate bud-primordium formation. Fig. 2, shallow tangential section of a 9-day-old hypocotyl, decapitated on the 5th day, showing epidermal cell, adjoining stoma, with transverse divisions which initiate bud-primordium formation. Fig. 3, shallow tangential section of a 15-day-old nondecapitated hypocotyl showing an epidermal cell with four transverse walls and a radial wall across each derivative cell. Often the radial division is preceded or followed by tangential division.

have engaged in, the first division in bud-primordium initiation do not appear very different from mature epidermal cells which divide radially in the course of diameter increase of the axis or from those which are becoming phellogenic or have just completed the first tangential division characteristic of phellogenic activity. RAUH (9) reports that, prior to the division which initiates bud-primordium vacuoles with cytoplasm. There is an increase in cytoplasm, but it is peripheral in the new cells and does not become strikingly evident until the derivative cells have become numerous and quite small by repeated divisions in several planes (figs. 3, 6).

In all material studied by us, the first division in initiation of the chain of divisions involved in bud-primordium forma-

tion is transverse (figs. 1, 2), as found by Eggers in 1937 (6). Generally, but not invariably, this is followed by one or several additional transverse divisions (figs. 1, 2) within the confines of the old wall. These early transverse divisions are soon or less simultaneously, some may divide tangentially, some radially, some transversely (figs. 4, 5, 6). After repeated divisions of the original cell derivatives, the cytoplasmic content of the new cells within the original cell has increased to
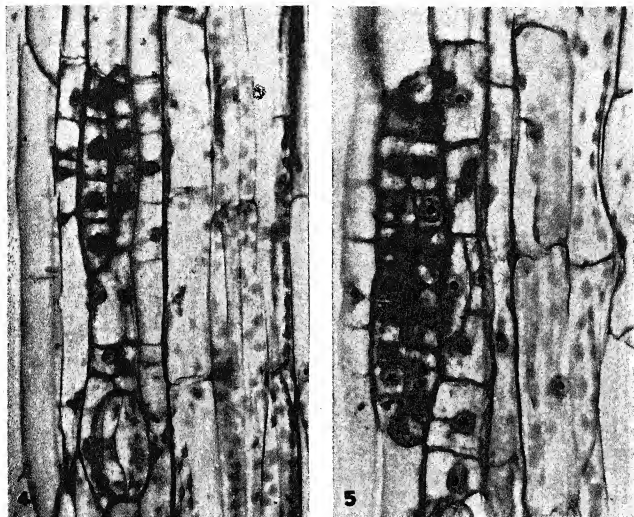

FIGS. 4–5.—Fig. 4, shallow tangential sections of a 9-day-old hypocotyl decapitated on the 5th day. The primordium had its origin in two adjoining pointed cells, which divided transversely more or less simultaneously. Following these divisions the flanking epidermal cells have undergone transverse division, and the epidermal cell adjoining the stoma has undergone transverse and radial division. Fig. 5, shallow tangential section of a 9-day-old hypocotyl, decapitated on the 5th day. The epidermal cells first to divide are seen almost in surface view. The original transverse divisions were followed by uneven transverse and radial divisions of the derivatives. The flanking epidermal cells have undergone division, transverse (left), and transverse and irregular radial (right). The lowest cell to the right adjoins a stoma.

followed by tangential, or by radial, divisions (fig. 3) or by a combination of these (fig. 6). The latter is possible because the new cells formed by the first and the immediately subsequent divisions do not always behave alike as to orientation of their spindles; hence, more such an extent and the nuclei are so evident that the meristematic area is easily detected in stained material. These meristematic sites are detectable after a few divisions with considerable certainty in longitudinal sections, but in transverse sections of the hypocotyl it is possible to

detect evidence of these events with certainty only after radial and tangential walls have come in, following the transverse wall or walls. This may account for the fact that Crooks (5) and Rauh (9) both missed the earliest stage of bud-primordium initiation and represented the two-division stage as a four-celled stage resulting from a radial and a tangential division. Crooks did not indicate which of these divisions he thought comes first. Rauh states that the radial generally, but not invariably, comes first. There is little, if any, cell enlargement during the first stages of transverse cell division (figs. 1, 2, 3). However, increase in volume of the derivatives becomes quite evident after radial and tangential divisions have taken place. The increase is due to increase in number and volume
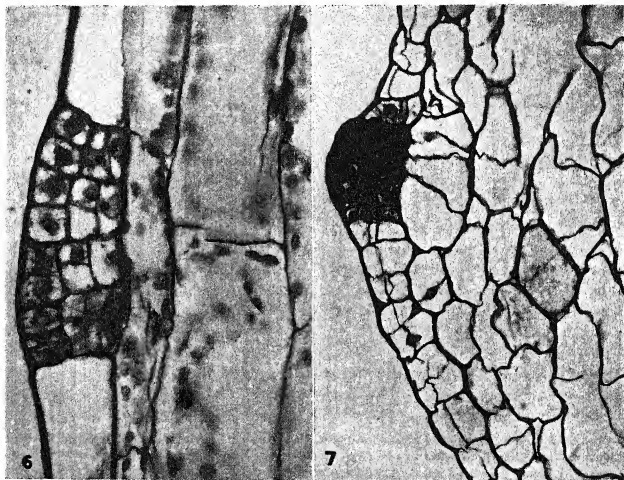
FIGS. 6–7.—Fig. 6, longisection of an 11-day-old hypocotyl decapitated on the 5th day. Three transverse divisions of an epidermal cell gave rise to derivative cells, which underwent two tangential divisions, with subsequent transverse and tangential division in only some of their derivatives. Many primordia in this stage also show radial division. The primordium has attained the "bulge" stage. No enlargement or divisions are evident in the hypodermal cells underlying it. In nondecapitated hypocotyls very few primordia reach this stage of development. Fig. 7, transverse section of a nondecapitated hypocotyl, 36 days old. Two epidermal cells have undergone a combination of transverse, radial, and tangential divisions and given rise to a primordium, which bulges above the surface. The cells of the hypodermis below the primordium are excessively enlarged and have divided radially. This might be only a response to tangential stretching or the beginning of the meristematic activity, which eventually may extend from the primordium to the endodermis and, in case the primordium becomes a bud, to the vascular elements of the main axis. The epidermis has become phellogenic and also shows some radial divisions. The other cortical cells have accommodated themselves to diameter increase of the axis by tangential stretching and radial division.

of nuclei, cytoplasm, and walls, and not to increase in size of vacuoles.

The subsequent stages of bud-primordium development are correctly described and figured by CROOKS (5) and RAUH (9). The epidermal cells adjoining the originally dividing one may become involved in chains of division like that of the cell first to divide (figs. 4, 5). If the events proceed further, the mass of epidermal derivatives begins to bulge above the surface of the hypocotyl (figs. 6, 7). We designate this stage the "bulge stage." Later, leaf primordia are formed which initiate the "bud" stage. The bulge is largely due to the mass of new cells formed, and not to vacuolar enlargement. The primordium, the subsequent bud, and the shoot are wholly derived from epidermal cells. The mature epidermal cells therefore are totipotent. Whether or not the derivatives of a single epidermal cell can develop a shoot we were not able to determine. In case that bud-primordium development proceeds beyond the bulge stage, the underlying hypodermal cells become meristematic, as first reported by BEALS (3) and later verified by CROOKS (5) and RAUH (9). After this, the meristematic activity extends progressively through the cortex and pericycle, until the phloem and xylem are reached. Later, the vascular derivatives of these meristems establish connections between the vascular elements of the bud and of the axis. The hypodermal and deeper cells involved generally enlarge before they begin to divide in the course of linking the bud with the vascular tissues of the main axis. These stages are not given in this paper because they are reported and excellently illustrated in the paper by CROOKS (5).

The course of events in bud-primordium initiation and development may be arrested at any stage. If stopped immediately after the first transverse division, it is not possible to identify positively the derivatives as prospective bud-primordium initials, even if one has a full-length surface view or a shallow tangential section of the cell involved.

A paper by BAIN (2) described the same course of events for the decapitated hypocotyl of cranberry. These identical findings for plants of different families raise the question of whether the course of cytogenesis in their hypocotyledonary bud formation is normal for other plants which initiate hypocotyledonary buds in the epidermis.

d) SITE AND TIME OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA

1) IN THE NONDECAPITATED HYPOCOTYL.—By the 5th day after planting or soaking of our May, 1939, material, the epidermal zone of the hypocotyl at, and immediately above, the ground line was made up of cells which either were elongating rapidly or had completed most of their longitudinal stretching. Almost invariably, among the epidermal cells of this zone in hypocotyls of this age, cells occur which are undergoing or have undergone the transverse divisions which initiate the chain of divisions that leads to formation of bud primordia. This finding was checked by examination of many other lots of nondecapitated and decapitated hypocotyls. We have never seen evidence of these divisions in 4-day-old seedlings. Initiation of this chain of divisions by epidermal cells of the normal, nondecapitated hypocotyl, therefore, begins between the 4th and the 5th days of development of the seedling. This is just at the close of the period of straightening of the hypocotyl and before the shedding of the seed coats and the enlargement of

the cotyledons. The zone in which these events occur is not determined by the soil line but by factors internal to the seedling. *Linum usitatissimum* does not form root buds.

Very often the epidermal cells adjoining the ends of the stomatal cells or cells near a stoma are the first to divide (figs. 2, 3). Short, but apparently fully elongated cells, pointed or not pointed at one or both ends, often are involved (figs. 3, 4), but division is not restricted to, or even more frequent in, these short cells. Often two adjoining short cells which occupy about the width of one of the longer epidermal cells are simultaneously the site of these divisions (fig. 4). They are easily detected in surface views of the epidermis. We have never seen a bud primordium in the upper half of a nondecapitated hypocotyl.

In the material of the May planting the largest number of bud primordia in a nondecapitated hypocotyl was found in a 15-day specimen. This hypocotyl grew in soil and bore thirty-five primordia on its lower half. Of these, thirty-two were in the early stages, and three had attained the bulge stage, but not the bud stage. An 11-day-old specimen bore fourteen bud primordia, and only one of these had attained the bulge stage. In a 7-, 6-, and 5-day-old hypocotyl, thirty-two, ten, and five bud-primordium sites, respectively, were counted. None had attained the bulge stage. Seven-, 6-, and 5-day-old hypocotyls grown in sand bore six, four, and three sites, respectively. None of these primordia had attained the bulge stage. There is great variation in the number of primordium sites in individual hypocotyls of the same age, and no readily demonstrable correlation between the number of bud primordia and the age of the hypocotyl has been found.

The oldest nondecapitated hypocotyl examined critically by us was fixed when 36 days old. It bore only six bud primordia, all restricted to the lower half. Three of these had attained the bulge stage. The hypodermal cells below these primordia had enlarged and possibly started (fig. 7) the chain of divisions incident to connecting a bud with the vascular system of the hypocotyl. Epidermal and hypodermal cells bordering the large and small primordia showed evidence of the stretching and divisions which accommodate these cells to diameter increase of the axis (fig. 7), and in the epidermis the tangential divisions of phellogenic activity were in evidence (fig. 7). While this material, like others examined by us, shows no evidence that bud primordia are isolated or sloughed off by periderm formation beneath them, we are not able to state unqualifiedly that this never occurs. Supposedly, the primordia, once formed, lie dormant in various stages of development in the nondecapitated hypocotyl.

Bud and shoot development occasionally proceeds from these primordia in non-decapitated hypocotyls of old plants, as reported by BURNS and HEDDEN (4) and as observed by us. BURNS and HEDDEN found such shoot development in 7 per cent of "uninjured" hypocotyls of plants grown in crocks which were kept moist. We noticed them in sand cultures under conditions of high nitrate nitrogen nutrition and bright sunlight. CROOKS did not record observation of such shoots. RAUH (9) stated that the perennial species of *Linum*, like many other plants which may develop subcotyledonary shoots without removal of the main axis (which he put into a class of facultative subcotyledonary shoot development), do so frequently in culture under favorable nutritive conditions but only rarely in their native habitats.

Whether the relatively few cases of development of these primordia into buds and even into shoots in the nondecapitated hypocotyl take place in absence of injury of any sort to the hypocotyl we were not able to determine. In only one young hypocotyl of the many nondecapitated ones of the May planting examined by us did we find a site which had progressed beyond the stage of epidermal involvement. In this instance the epidermal derivatives bulged above the surface of the 7-day-old hypocotyl, and the hypodermis and several layers of the cortex had become meristematic. While this hypocotyl was free of any grossly detectable wound, microscopic examination revealed a puncture, which extended into the cortex of the axis above the site of the primordium. It is possible, therefore, that some of the hypocotyledonary shoots which develop occasionally from nondecapitated hypocotyls result from wounds of the hypocotyl which are not grossly detectable.

2) In the decapitated hypocotyl.— Burns and Hedden (4) reported that the seedlings of *L. usitatissimum*, if decapitated immediately below the cotyledons when 2–3 cm. high, produce buds which are not restricted to the base of the hypocotyl and range in number from one to sixty or more per hypocotyl. They also found that the age of the hypocotyl at time of decapitation is a factor in bud formation both as to its frequency and as to its site. Crooks (5) reported that seedlings decapitated before they are 10 days old produce buds in 6–8 days and that 60 per cent of plants severed below the cotyledons when 60 days old died without producing buds. He found that all hypocotyls decapitated when not more than 10 days old produce five to twenty buds on the remaining part of the hypocotyl, even when the cut was made as

low as a few millimeters above the soil line. Eggers (6, 7) corroborated and enlarged upon these findings and found that, in general, decapitation on the 10th day after emergence of the seedling results in maximum survival of plants and maximum bud production under the various conditions of nutrition tested at different times of the year. In the course of our studies we found that decapitation at any level and at any age up to 60 days, and occasionally even more, could be followed by grossly detectable bud development. With increasing age and severe external conditions, such as high temperature, intense light, low humidity, and poor ventilation, an increasing number of hypocotyls die with or without development of buds and with a decrease in the number of buds developed per plant.

Crooks (5) showed that the observation made by Burns and Hedden (4), that not all buds start grossly detectable development at the same time, is due to the fact that, while buds on one part of the hypocotyl are large enough to show small leaves, many others are in earlier stages of development. He found that after eight to ten buds begin to show small leaves, one bud usually outgrows all the others. This bud may be at any level in relation to the others, and it is not necessarily the first to have made its gross appearance. Occasionally, more than one bud continues development, but, in time, one gets the ascendancy. Our observations are in agreement with these findings by Crooks.

Burns and Hedden (4) reported that, following decapitation of the hypocotyl, its epidermal cells become rich in chloroplasts. We did not find this to be the case. Crooks reported that the swelling of the hypocotyl noted by Burns and Hedden following decapitation is due to formation of large intercellular spaces. We

were able to corroborate that finding.
CROOKS reported that the hypocotyle-
donary bud is initiated by division of an
epidermal cell and that this division is
quickly followed by a second at right
angles to it, producing a four-celled
stage, with a radial and a tangential wall.
RAUH (9) reported the same for *L.
usitatissimum* and for *L. tenuifolium*.
We found that the mode of bud-primor-
dium initiation in the decapitated hypo-
cotyl is the same as in the nondecapi-
tated hypocotyl, i.e., it begins with a
transverse division of an epidermal cell
which is elongating rapidly or, more gen-
erally, has completed its elongation (fig.
2). Here, too, the cells which divide
transversely are not necessarily the most
elongated ones. In fact, many of them
are very short. As in nondecapitated
hypocotyls, epidermal cells adjoining the
ends of stomatal cells or near the stomata
seem to be the most frequent sites of
these divisions (fig. 2).

While no epidermal cells in the upper
half of the nondecapitated hypocotyl
initiate bud primordia, the cells in this
region of the decapitated hypocotyl initi-
ate bud primordia in great abundance.

In our May, 1939, material, decapi-
tated immediately below the cotyledons
when the seedlings were 5 days old, a
hypocotyl 2 days after decapitation (7-
day-old material) bore twenty-one sites
of bud-primordium initiation in its lower
half. These primordia were in about the
same stage of development as those in a
nondecapitated hypocotyl of the same
age. Another specimen grown in sand
bore sixteen sites, all restricted to the
lower half of the hypocotyl. None of the
primordia had attained the bulge stage.

By the 4th day after decapitation,
striking differences were evident between
nondecapitated and decapitated hypo-

cotyls (all 9 days old). As pointed out
before, none of the nondecapitated hypo-
cotyls bore bud-primordium sites in the
upper half of the hypocotyl, and none of
the primordia had advanced past the
bulge stage. In contrast, a hypocotyl
grown in soil, 9 days old and 4 days after
decapitation, bore fourteen sites in its
upper half and thirty-five in its lower
half. None of the primordia of the upper
half had attained the bulge stage, but one
in the lower half had. A specimen of the
same age but grown in sand bore thirty
sites in its upper half and twenty-three in
its lower half. Eight of the former sites
bore primordia in the bulge stage, and
one bore a bud stage. None of the pri-
mordia of the lower half had attained the
bulge stage. The findings for hypocotyls
10 days old (6 days after decapitation)
are of the same order except that more
sites bore advanced stages. The hypo-
cotyl grown in soil bore nine sites in its
upper half and thirty-nine in its lower
half. In three of the former the primordia
were in the bulge stage and in two, in the
bud stage. In twelve of the sites in the
lower half, the primordia had reached the
bulge stage and in one, the bud stage. A
hypocotyl of this age grown in sand bore
thirteen sites in its upper half and twenty
in its lower half. In two of the former, the
primordia were in the bulge stage and in
four, in the bud stage. In two sites in the
lower half, the primordia had reached the
bulge stage and in two, the bud stage.
No sample of a hypocotyl 15 days old (10
days after decapitation) grown in sand
was sectioned. The soil-grown sample
bore fourteen sites in its upper half and
sixteen in its lower half. In two sites in
the upper half the primordia had reached
the bulge stage; five had reached the bud
stage; and one had become a shoot,
2 mm. long. It was located near the top

of the decapitated hypocotyl. In two sites of the lower half, the primordium had reached the bulge stage.

Examination of many other nondecapitated and decapitated hypocotyls indicates that, on the average, the decapitated hypocotyl bears more bud primordia in its lower half than the nondecapitated. However, variation in the number of bud primordia and buds per hypocotyl is so large that an extensive statistical study would have to be undertaken to determine whether or not decapitation increases the number of primordia in the lower half of the decapitated hypocotyl. Occasionally, one finds a decapitated hypocotyl which does not bear any primordia in its upper half.

In order to determine whether or not the absence of buds and shoots from the upper half of hypocotyls decapitated late is due to noninitiation of primordia by this region or to nondevelopment of primordia present at time of decapitation, we examined the hypocotyls of plants which had been decapitated on the 36th day and collected and fixed on the 46th day. As in the nondecapitated hypocotyls of this lot recorded above, no primordia were found in the upper half of the axis, and fifteen were found in the lower half. This corroborates our general finding that the epidermal cells of the upper half of the hypocotyl, and probably of the lower half, in time lose their capacity to initiate bud primordia but that the capacity to develop the bud primordia present into buds and shoots may be retained up to the 60th day, and even longer.

During the 10 days following decapitation of this 36-day-old plant, one bud primordium had progressed to the stage of leaf-primordium formation and the hypodermis and three additional layers

of the cortex had become meristematic in the manner described by CROOKS (5). This is a slow rate of development compared with that of primordia of hypocotyls decapitated between the 5th and the 10th days. As noted before, when hypocotyls are decapitated later than the 10th day, the chances of their survival are greatly reduced. If permitted to grow, this bud in the 36-day-old material probably would have established connections with the vascular tissues and grown into a shoot, provided the hypocotyl did not die. As in the material collected on the 36th day, there was no evidence that the increased phellogenic activity from the 36th to the 46th days had extended below the sites of even the youngest bud primordia. We have found no instance of phellogenic origin of hypocotyledonary buds.

Discussion

Some of the current interpretations of the hypocotyledonary bud situation in *L. usitatissimum* are inadequate because they do not consider the fact that epidermal cells of the lower half of the normal, nondecapitated hypocotyl undergo the first stages of bud-primordium formation and that these primordia occasionally develop into buds and shoots. BURNS and HEDDEN (4) attempted to explain their observations that a nondecapitated hypocotyl occasionally develops a shoot at its base and that decapitated hypocotyls, especially those decapitated when old, frequently develop shoots only at the base, by assuming that the base of the hypocotyl is "especially predisposed" to bud formation and that it retains its ability to form buds longer than the rest of the hypocotyl.

In his studies of substitute shoot and root formation (*Ersatzbildung*) by vari-

ous species of *Linum*, NEUBERT (8) found it necessary to distinguish between (1) replacement of the lost member in a similar site (*Wiederbildung*); (2) *de novo* formation of a member at the site of loss (*Neubildung*); and (3) development of a dormant member which replaces the lost member (*Neuentfaltung*). Not being aware that some of the hypocotyledonary shoots of *L. usitatissimum* are the result of development of a dormant member, he considered all the hypocotyledonary shoots of this plant to be examples of replacement of a lost member in a similar site.

RAUH (9) recognized three types of subcotyledonary shoot formation, which he designated (1) obligate, (2) facultative, and (3) regenerative. He used the plant's dependence or nondependence for survival on development of subcotyledonary shoots as a criterion to define and differentiate the obligate and facultative types. In defining and differentiating the regenerative type, he did not use this criterion but adopted the ability of the plant to form buds after removal of the main axis as the criterion. Had he not shifted criteria, he would have been able to consider the regenerative type a subtype of the facultative type. RAUH ignored BURNS and HEDDEN's report that *L. usitatissimum* occasionally produces subcotyledonary buds and shoots without decapitation of the hypocotyl, and he was not aware of the presence of bud primordia in the lower part of the normal hypocotyl of this plant. Accordingly, he classified its hypocotyledonary shoots as regenerative.

The system of classification formulated and the criteria adopted by RAUH can be employed more logically and consistently than he has done. This may be accomplished by retention of the obligate and facultative types and by division of

the latter into at least two subtypes. The first subtype may be defined so as to comprise those subcotyledonary shoots which develop without removal of the main axis, and the second so as to comprise those which develop after removal of the main axis. Subtype 2 needs division into group A, comprising those shoots which develop from buds (primordia) laid down before removal of the axis, and group B, comprising those shoots which develop from buds (primordia) laid down after removal of the axis. According to this modified system, some of the hypocotyledonary shoots of *L. usitatissimum* belong to the first subtype, some to the second, and, of the latter, some belong to group A, some to group B. Reinvestigation of the other plants with subcotyledonary shoots which have been classified by RAUH may reveal that in them, too, the subcotyledonary shoot situation is more complex than appears superficially.

Our findings indicate that the epidermal cells of the hypocotyl must be in a definite stage of development for initiation of the chain of divisions which characterizes bud-primordium formation. This stage apparently is realized while the epidermal cells are in the final stages of elongation and, for a time, after this elongation has ceased. The epidermal cells which are sites of the first transverse divisions of bud-primordium formation do not experience greater enlargement prior to the first division than do their neighbors which do not divide, nor do they show marked increase in cytoplasmic content. Our findings also indicate that initiation of this chain of divisions by cells in the proper stage of development may begin in the presence of the cotyledons and epicotyl on the 5th day in the development of the seedling but that after a short time it is inhibited,

so that in intact seedlings bud primordia are found only in the lower half of the hypocotyl, whereas in the decapitated young hypocotyl they occur at all levels of its remainder.

Further development of the early stages of bud primordia also is affected by the cotyledons and epicotyl, so that, except occasionally, it does not progress beyond the stage of epidermal involvement and stops short of leaf-primordium formation. In intact plants the primordium only rarely develops into a bud, and the bud develops into a shoot even more rarely.

These inhibitions of the capacity to begin and to continue the chain of divisions which characterize bud-primordium initiation and development are not instances of a generalized inhibition of meristematic capability of epidermal cells of the hypocotyl. At the time of these specific inhibitions of bud-primordium initiation and development, these cells still are capable of radial division and even more of the tangential divisions which characterize phellogenic activity.

Summary

1. Transverse division of an epidermal cell is the first identifiable stage in initiation of a hypocotyledonary bud primordium in *L. usitatissimum* L.

2. Generally, a second transverse division in one or both of the daughter cells, but not infrequently either a radial or a tangential division, is the second stage.

3. These divisions are not preceded by grossly detectable increase in size of the cells involved, nor is there marked increase of the cytoplasmic contents of these cells prior to or immediately following these divisions.

4. These events begin in epidermal cells of the lower half of the hypocotyl between the 4th and 5th days of the developing seedling. Development of primordia of the lower half of the hypocotyl into buds, and of these buds into shoots, rarely occurs in the normal, nondecapitated hypotocyl.

5. Upon decapitation of the hypocotyl, one to many of the bud primordia in the lower half of the hypocotyl may develop into buds, and one or several of these may develop into shoots.

6. The epidermal cells of the upper half of the hypocotyl also are capable of initiating bud-primordium formation but do not do so unless the hypocotyl has been decapitated. Following decapitation between the 3d and 15th days after emergence of the seedling, a great number of primordia may be initiated by these epidermal cells. Many of these primordia may develop into buds, and one (occasionally several) of these buds may develop into a shoot. After a shoot from either the lower or the upper half of the hypocotyl has established dominance, the development of the other shoots ceases or proceeds very slowly.

7. The epidermal cells apparently must be in a definite stage of development for initiation of the chain of divisions which characterizes bud-primordium formation. This stage apparently is realized while or after the epidermal cells are in the final stage of elongation and persists for a time after this elongation has ceased. It is first attained in the zone of general elongation in the lower half of the hypocotyl and proceeds upward as this zone moves up along the hypocotyl.

8. Presence of the cotyledons and epicotyl does not inhibit initiation of bud primordia in the lower half of the hypocotyl but does inhibit it in the upper half. Generally, the presence of these organs and, later, of the epicotyl alone inhibits

development of the bud primordia of the lower half of the hypocotyl into buds and of the occasional bud into a shoot.

9. Generally, if hypocotyls are decapitated after the 20th–30th days after emergence, no bud primordia are formed in the upper half of the hypocotyl, but some of the primordia present in the lower half may develop into buds, and, of these, one or rarely two may develop into a shoot.

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THE RELATION OF PHOTOPERIOD TO THE BORON REQUIREMENT OF PLANTS[1]

ROBERT MacVICAR AND B. ESTHER STRUCKMEYER

Introduction

In the course of experiments on the boron nutrition of plants, it was observed that plants responding to short photoperiod exhibited diminished response to boron deprivation. Warington (9) had noted in *Vicia faba* and other plants that there was a delay in producing symptoms of boron deficiency during shortened days and had attributed such effects to differential rates of growth. Dufrenoy (3) had also observed, in a study of the effect of boron on the development of the root-nodule system in the same plant, that artificially extending the photo-

[1] Published with the approval of the Director of the Agricultural Experiment Station.

period during February and March hastened the development of symptoms of boron deficiency. Since the present study was undertaken, Skok (7) has also reported that, under short photoperiods, the symptoms of boron deficiency in the radish were less marked.

Methods

Soybeans (var. Biloxi and Manchu no. 3) were selected because of their known response to photoperiod (2) and because previous work had shown them to have a reasonably high boron requirement when grown in long days. Seeds were disinfected with $CaOCl_2$ and germinated in silica sand. After the development of the

first true leaves, the seedlings were transferred to leached silica sand in varnished, 10-inch clay pots. Five plants were planted in each pot, and there were four replications of each treatment. Approximately 500 ml. of the following nutrient solution was added four times weekly:

	Experiment 1	Experiment 2
K_2HPO_4.....	0.0012 Molar	0.0012 Molar
KH_2PO_4.....	.0018	.0018
$Ca(NO_3)_2$....	.0084	.0042
$CaCl_2$.......	.0011	.0011
$Mg(NO_3)_2$...	.0042	.0021
$MgSO_4$......	.0011	.0021
NH_4NO_3.....	0.0042	0 0021

Boron was added at a 0.5-p.p.m. level. Other micronutrient elements were those recommended by HOAGLAND and ARNON (4). All pots were thoroughly leached with distilled water at least once weekly to remove any unbalanced solution.

All plants were grown under normal greenhouse conditions of light intensity, humidity, and temperature. During the summer months the houses were whitewashed. In experiment 1, plants grown in normal day length were placed in a more exposed house having a somewhat higher sunlight intensity, 1400 foot candles as compared with 1000 foot candles in those houses under controlled lengths of photoperiod. The short photoperiod was varied by manually operated screens. Incandescent light bulbs, with an automatically controlled time switch, provided extended length of photoperiod from near sunset until midnight.

Samples were customarily taken in the early morning before 9:00 A.M. to minimize variations in water content. After being removed from the pot, the tops and roots were separated, the roots were freed of sand and blotted dry between sheets of absorbent paper, and the parts were weighed and immediately transferred to drying ovens. After remaining in these ovens under a constant stream of air at 60° C. until reaching a constant weight, they were re-weighed, ground to a fine powder in a Wiley mill, and stored for analysis.

Samples for microscopic examination were taken at the fourth internode from the tip and fixed in formalin-aceto-alcohol. These were dehydrated in *n*-butyl alcohol and imbedded in paraffin. The stems were cut $12\,\mu$ in thickness on a sliding microtome. The sections were stained with iron-alum-hematoxylin and safranin.

Ash was determined on the 60° air-dry samples by combustion in a thermostatically controlled muffle furnace at 500° C. Boron was determined by the quinalizarin procedure of BERGER and TRUOG (1), as modified in this laboratory (6).

Results

EXPERIMENT 1.—Soybean seedlings (Manchu no. 3) were transferred to pots on April 17, 1941, and on April 19, 1941, and were placed in differential photoperiods of 9 and 17–18 hours, respectively. By May 10, boron-deficient plants in long days were showing restricted growth, but there was no detectable difference between plus and minus boron cultures in short days. A month after being placed in different photoperiods, boron-deficient plants in long day had developed typical symptoms, including fragility of the leaves and petioles, necrosis of apical meristematic tissue, and reduced secondary-root formation. Boron-deficient plants in short day, however, continued to develop in a nearly normal manner for plants which fruit in this photoperiod. Blossom primordia had appeared in all treatments in short and normal days; long-day plants continued to remain vegetative throughout the en-

tire length of the experiment. At the time of harvesting (June 4, 1941), boron-deficiency symptoms were very severe in long-day cultures and moderately severe in normal day length, while plants in short day appeared essentially normal. Data with reference to growth, per cent dry matter, top/root factor, and ash and

boron content are presented in table 1. Figure 1 shows the appearance of the plants on May 21.

EXPERIMENT 2.—This experiment was essentially a duplicate of the previous one, except that the short-day variety, Biloxi, was used. Plants were transferred to differential day lengths on October 21,

TABLE 1

EXPERIMENT 1: EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT
OF NORMAL AND BORON-DEFICIENT MANCHU SOYBEANS

TREATMENT	AVERAGE DRY WEIGHT OF TOPS (GM.)	DRY MATTER (PER CENT)	TOP/ROOT	LEAF TISSUE	
				Ash*	Boron†
5-21-41 sampling					
Long day plus boron	1.24	13.7	4.2	12.8	63.8
Long day minus boron	0.56	14.3	3.7	11.6	13.7
Normal day plus boron	1.16	20.7	2.0	8.6	46.8
Normal day minus boron	1.25	21.4	2.7	8.7	16.2
Short day plus boron	0.59	13.7	3.5	13.4	56.8
Short day minus boron	0.66	15.1	3.7	12.5	21.1
6-4-41 sampling					
Long day plus boron	3.36	17.5	6.7	11.6	61.2
Long day minus boron	1.18	17.9	4.0	11.3	16.8
Normal day plus boron	3.10	22.5	2.9	11.8	88.7
Normal day minus boron	2.80	27.2	2.3	8.8	14.6
Short day plus boron	1.69	20.9	4.9	11.3	48.5
Short day minus boron	1.92	25.3	4.1	11.7	14.5
6-24-41 sampling					
Long day plus boron	4.76	23.2	7.8	10.4	58.9
Long day minus boron	1.01	20.6	4.8	13.6	21.5
Normal day plus boron	9.90	21.4	6.4	9.9	62.1
Normal day minus boron	4.83	26.4	4.0	10.3	19.8
Short day plus boron	1.15	25.0	5.9	12.4	71.0
Short day minus boron	1.83	26.6	5.6	11.0	23.8

* In per cent on a moisture-free basis.
† In micrograms per gram of dry tissue.

1941. A month later, initial symptoms of boron deficiency had appeared in cultures in long photoperiod. By December 23 the typical fragility of the leaves, petioles, and upper internodes was pronounced; necrosis of meristematic tissue had occurred (although somewhat less extensively than in the Manchu variety); and secondary root development was restricted. Plants grown in short day produced fruits, regardless of the level of boron supplied. Data concerning the growth of these plants are presented in table 2.

ANATOMICAL OBSERVATIONS

The anatomical structure of soybean plants (Manchu no. 3) grown with and without boron in long days was quite different (figs. 2, 3). The normal stem was in the budding stage when sampled, and it exhibited the anatomical characteristics typical of budding stems, namely, a thickening of the walls of the vascular tissue and a decrease in cambial activity (8) (figs. 2, 6). The plants grown without boron remained in the vegetative state and showed an unusual type of stem anatomy. The phloem region was about four times the diameter of the normal stem (figs. 3, 8). The most conspicuous difference was the increased number and size of the phloem cells and ray cells resulting from abnormal cambial activity and some proliferation. Numerous sieve tubes and companion cells had been formed, and considerable necrosis had occurred in these regions. The ray cells especially had stretched in a radial direction and increased in size.

There was also a greater number of xylem elements in the boron-deficient stem, xylem parenchyma being formed before the cambium became inactive (fig. 8). Cells in the region of the pericycle showed considerable tangential

stretching, radial division, and necrosis. The fiber cap had become separated into groups of fibers by internal pressure of the enlarging cells. The fibers themselves remained unchanged. There was an increase in cell size and some tangential division in the endodermis. The collenchyma cells of the cortex were much larger and thinner walled in the stems of plants grown without boron. The area of
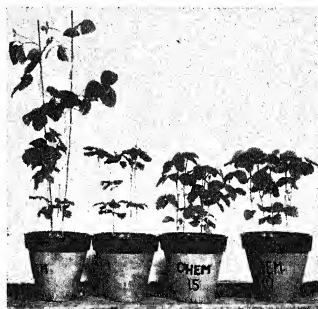

FIG. 1.—Plants of soybean (Manchu no. 3), grown in short and long days. A, plant grown in long photoperiod with boron; B, plant grown in long photoperiod without boron; C, plant grown in short day with boron; D, plant grown in short day without boron; photographed May 21, 1941.

the pith region was somewhat less in the stems of these abnormal plants.

The anatomy of the stems of plants grown with and without boron but in short days was not greatly different. Plants grown with boron had fruits and showed the characteristic anatomy of fruiting stems, such as thickening of the walls of the vascular tissue and absence of cambial activity (fig. 4). Plants grown without boron were in the blossoming state. The effects of boron deficiency were apparent in the stem (figs. 5, 7) but not so conspicuously as in plants grown

in long days (figs. 3, 8). The phloem parenchyma cells and ray cells showed some enlargement, but it was much less apparent than in plants grown in long photoperiod. Sieve tubes and companion cells were not greatly affected, and necrosis was not present. There was some tangential division in the endodermal

TABLE 2

EXPERIMENT 2: EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT OF NORMAL AND BORON-DEFICIENT BILOXI SOYBEANS

Treatment	Average dry weight of tops (gm.)	Dry matter (per cent)	Top/root
	11-24-41 sampling		
Long day plus boron.....	1.39	13.8	6.7
Long day minus boron...	1.10	12.8	5.7
Short day plus boron....	0.98	14.8	4.6
Short day minus boron..	0.93	14.8	4.9
	12-23-41 sampling		
Long day plus boron....	3.53	15.6	5.0
Long day minus boron...	2.22	10.9	4.9
Short day plus boron....	1.75	21.6	5.0
Short day minus boron..	2.01	22.8	4.5

cells, the fibers remained normal, and the cortical cells were larger than those in the normal stem (fig. 7). The great increase in number of cells due to cambial activity and proliferation in plants grown in long days was markedly less in plants grown in short days but also without boron.

Discussion

An examination of the data presented in table 1, together with the gross appearance of the plants (fig. 1) and the more detailed anatomical structure of the stem (figs. 2–5), reveals that the symptoms of boron deficiency were far more severe in long photoperiods than in short day lengths, which induce fruit formation. This observation was confirmed with the Biloxi variety, although the differences in gross weight of tissue produced were less marked.

It will be noted that the severity of symptoms in Manchu was not correlated with the boron content of the tissue. Thus the boron content of the leaf of boron-deficient plants, in long and short days at the end of experiment 1, was found to be 21.5 and 23.8 µg per gram, respectively. In one group of plants, this level was adequate for essentially normal functioning; in the other, severe symptoms of deprivation resulted. The difference in boron content, therefore, was eliminated, and a reduction in boron requirement appeared to have occurred.

In boron-deficient plants in long days (fig. 8) there was an abnormal cambial activity, as evidenced by an increased amount of phloem, ray, and xylem tissue. In short photoperiod, boron-deficient plants did not exhibit such marked cambial activity. The question arises as to whether the reduced cambial activity produced by the shortened photoperiod (fig. 7), which hastened blossom induction, was responsible for the reduced need for boron. The development of the abnormal cambium in boron-deficient plants of cabbage also had been observed by JOLIVETTE and WALKER (5).

This relationship between the boron requirement and photoperiod re-emphasizes the importance of controlling the environmental conditions in studies involving the metabolism of plants. Experimental studies dealing with boron should be conducted under similar photoperiods. In attempting to produce boron
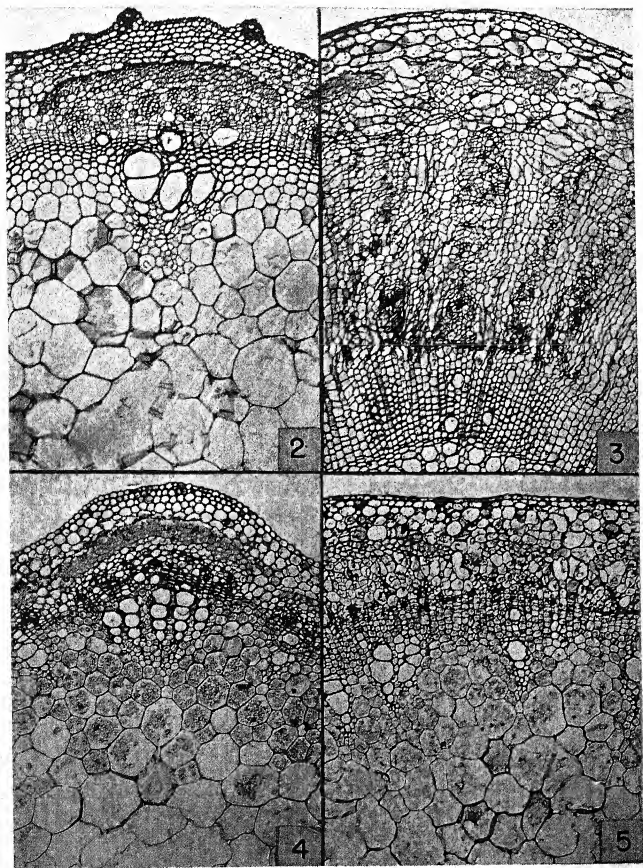
FIGS. 2–5.—Cross-section of fourth internodes of soybean (Manchu no. 3). Fig. 2, early-blossoming plant with boron in long photoperiod. Fig. 3, plant without boron in long photoperiod showing increased phloem, ray, and xylem tissues from cambial activity and proliferation. Necrotic areas also apparent. Fig. 4, fruiting plant in short photoperiod; the inactive cambium and thick-walled vascular tissue are characteristic of fruiting stems. Fig. 5, short photoperiod without boron. Compared to figure 3, only slight increase in phloem and ray tissue.
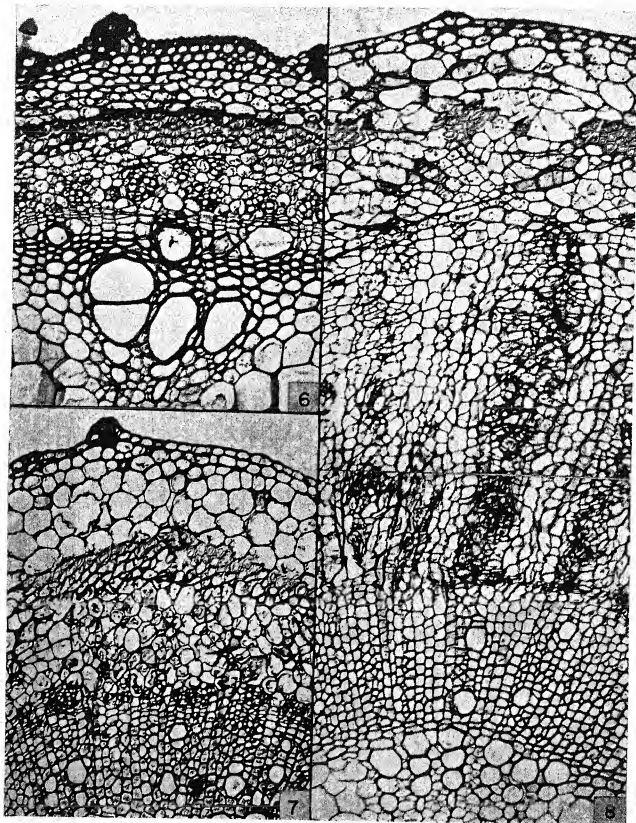
FIGS. 6-8.—Cross-section of soybean plants (Manchu no. 3). Higher magnification of figures 2, 3, and 5. Fig. 6, normal structure of plants grown with boron and long photoperiod. Fig. 7, relatively slight distortion from lack of boron in short days. Fig. 8, abnormal structure resulting from lack of boron in long days.

deficiency in plants exhibiting photoperiodic sensitivity, moreover, it would appear desirable to use long-day lengths.

Summary

1. The intensity of boron-deficiency symptoms in soybeans has been found to be much more severe in long than in short days.

2. The reduction was due to a diminished boron requirement rather than to changes in the absorption of boron. The boron content of plants exhibiting severe symptoms of deprivation (long photoperiod) and plants showing no gross evidence of deficiency (short photoperiod) was essentially the same.

3. Histological examination of the stems confirmed this observation. There was little cellular disorganization in minus-boron plants produced in short photoperiod. Boron-deficient plants in long photoperiods, however, exhibited abnormalities. There was a marked increase in vascular tissue resulting from abnormal cambial activity. Necrotic areas in the region of the phloem were conspicuous.

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RADIAL GROWTH OF TREES AT DIFFERENT ALTITUDES

R. F. DAUBENMIRE

Introduction

Through the series of vertically stratified climates on the slopes of the Rocky Mountains, drought becomes an environmental factor of increasing importance in a downward direction, while low summer temperatures play an ever more critical role upward. In the light of existing information, these aspects of climate appear to set the limits of vertical distribution of most of the tree species (1). The present investigation is an attempt to ascertain the extent to which this climatic gradient affects the course of radial growth in certain native coniferous trees growing near their upper and lower altitudinal limits.

The study was conducted at Thatuna Ridge, the summit of which is located 12 km. northeast of the town of Moscow, Idaho. Because this ridge is oriented in an east-west direction and rises over 600 meters above the adjacent basal plain, it was possible to compare trees growing at low altitudes on the south slope with others located at high altitudes on the north slope and to obtain wide differences in climate. Because of the lateness of the opening of roads in spring and their impassibility after the rainy season begins in autumn, the study included only the 5-month period between April 19 and October 17, 1942.

Four trees were studied at each station, and a low- and a high-altitude station were established for each of the following species: *Pinus ponderosa* Dougl., *Abies grandis* Lindl., *Larix occidentalis* Nutt., and *Thuja plicata* Donn. The REINEKE dendrometer (3) was used in this investigation; and, although the apparatus proved to have an inherent error of at least ±0.002 inch, the use of aver-

ages based on four individuals overcame most of the errors in measurement. Another source of unavoidable error was the fact that the trees were measured at different times of day; and, consequently, diurnal fluctuations in radius, which are due to changes in the degree of tissue hydration, affected the measurements. This error, however, was reduced to insignificance by the facts that measurements were made only at intervals of a week or more and so were submerged by radial changes of greater magnitude and that the measurements of all trees under comparison were taken within a few hours, so that errors were approximately equal. The smoothness of the curves obtained and the fact that most radial changes were correlated with weather changes provide evidence that neither of the above sources of error can account for any of the conclusions.

The most relevant weather data available for the summer of 1942 were recorded at a co-operative station of the United States Weather Bureau located at Moscow, which is situated on the prairie-covered basal plain below the altitude of the lowest trees studied. It is believed that these data, which are summarized in figure 1, are sufficiently indicative of weather trends on the ridge to be very useful in interpreting observations.

In figure 1 is shown the quantity of water falling at each precipitation, when this amount exceeded 2.54 mm. During the rainy season precipitation undoubtedly increases with altitude on the ridge, but a series of five rain gauges maintained by the writer from July 17 to September 20, 1942, showed that there was no altitudinal gradient on the ridge during that period. In accordance with the normal

character of the climate, rainfall practically ceased in early summer, so that the warm season was also a season of cumulative drought. The significance of this dry season is to be reckoned in terms of high transpiration stress, to which at low altitudes is added the effect of desiccation of those soil horizons which contain the upper parts of the root systems.

Weekly means of the daily maximal and minimal temperatures, and weekly evaporation from a free water surface are shown in figure 1. Too much weight cannot be given the last statistic, for the Weather Bureau station is surrounded by lawns that are irrigated with sprinklers, and the latter are sometimes set so close to the instruments as seriously to affect the records.

PINUS PONDEROSA

Because of its relatively low altitudinal distribution, *P. ponderosa* is not found at high altitude on the north side of Thatuna Ridge, and for the upper group of trees a station in the arborvitae-hemlock zone[1] at an altitude of 1065 meters on the south slope was used. The lower station was in the ponderosa pine zone on the same slope, but at an altitude of 838 meters.

At both stations rapid radial growth was in progress by April 24, and, from the steepness of the curve (fig. 2), it may be estimated that growth had begun by at least mid-April. Until late May, the rate of growth was distinctly less at the higher station, presumably because of the lower temperature; but, starting in late May, the growth rate declined at the lower station and increased above. In early July a period of above-average heat

[1] The four major forest zones of northern Idaho are, in order of increasing altitude, ponderosa pine zone, Douglas fir zone, arborvitae-hemlock zone, and spruce-fir zone. The forests of these zones have been briefly characterized elsewhere (1).

and dryness (fig. 1) completely arrested growth at the lower station but had practically no effect above. There followed a week of moderated temperature with showers, and, in response to this change, the normal rate of growth was resumed at the lower station and slightly increased at the upper. Immediately following this last series of showers, maximal temperatures again rose to high levels and the long dry season began.

During the latter half of summer the trees at the lower station suffered considerable shrinkage. This was progressive
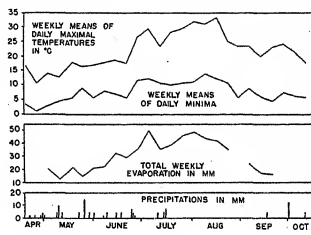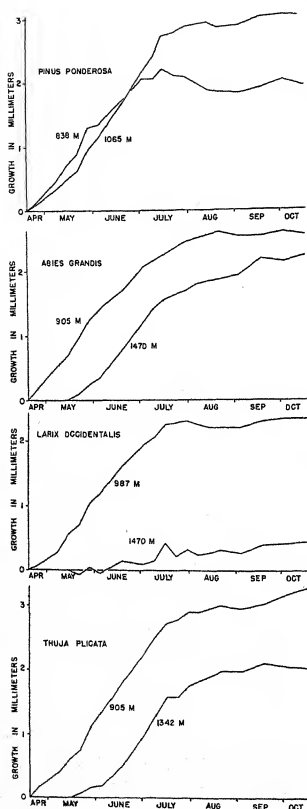
FIG. 1.—Summary of weather data taken at Moscow, Idaho, from April 19 to October 17, 1942.

until early September, when a period of cool weather, during which a small shower fell at Moscow, allowed recuperation to begin. Assuming that the evaporation data for Moscow are correct, this rain did not have an appreciable effect upon the rate of evaporation, which had already declined strongly in accordance with reduced temperatures (fig. 1). Either rainfall was considerably greater on the ridge and, in consequence, definitely reduced evaporation there, or the shower benefited the plants in some way other than through increasing relative humidity. The rain of October 1 also stimulated enlargement, but the effect was only temporary, and, during the rela-

FIGS. 2–5.—Fig. 2. radial changes in two groups of trees of *Pinus ponderosa* growing at different altitudes. Fig. 3, same for *Abies grandis*. Fig. 4, same for *Larix occidentalis*. Fig. 5, same for *Thuja plicata*.

tively dry period that followed, dehydration once again set in.

At the upper station, growth continued a month later in the summer, and shrinkage was of relatively minor extent and duration. Apparently, the more favorable soil-moisture conditions there enabled the trees to take advantage of the highly efficient midsummer temperatures for a longer period, and then subsequently allowed them to retain their diametral gains. The greater increment at the upper station is in accord with the fact that *P. ponderosa* normally grows to larger size at altitudes higher than those at which it is the sole dominant of the forest.

ABIES GRANDIS

Owing to the condition of the road, the upper station for *Abies* could not be visited until May 15. From this date until June 5 the trees at the upper station grew more slowly than those at the lower (fig. 3). Late-summer shrinkage was slight at the lower station, but no shrinkage was observed above that could be definitely attributed to drought. The greater net growth at low altitude is probably attributable to the fact that temperatures were more favorable for growth, and at the same time the trees were at an elevation high enough that the water balance was not an important limiting factor.

LARIX OCCIDENTALIS

The two stations for *L. occidentalis* were essentially identical with those for *Abies*, but there was much greater difference between the two groups of individuals in *Larix* (fig. 4). In this species the trees at high altitude made very slow growth throughout most of the period of observation, but the radii were subject to alternate expansion and contraction

to a degree not observed in any other group of trees. Likewise, at the lower station the behavior of *Larix* was in marked contrast with *Abies* growing there. In *Larix*, shrinkage began about 3 weeks earlier and eventually became more pronounced; but this species also responded more quickly to the mid-September shower.

THUJA PLICATA

In common with the other species, the trees of *Thuja* grew more slowly at the higher station in spring and early summer (fig. 5). At the lower station there were two distinct growing seasons, which were interrupted by a period of quiescence that included slight shrinkage in late August. The second growing season began with the first showers of the rainy season and lasted until at least October 17. At high altitude, growth was essentially complete by mid-September, and the dry late-summer weather had less effect upon the radii.

Discussion

In all instances the trees at low altitude on the south slope of Thatuna Ridge were growing by April 19, 1942, and those at high altitudes on the north slope were growing by mid-May. Although the observations did not begin early enough to ascertain whether or not the dates of beginning of growth differed among the habitats, the data consistently show that the growth rates in spring and early summer are more rapid, the lower the elevation. In *P. ponderosa* this difference was pronounced, with a vertical distance of only 227 meters.

The cumulative effects of dry summer weather were felt at all altitudes on Thatuna Ridge, but at low altitude the effect was always earlier and more pronounced. In all four species the individuals at the lower stations shrank in late summer, and shrinkage was observed in *Pinus* and *Larix* at the upper stations as well.

Shrinkage in *Pinus* at low altitude was so severe that by October 17 the trees had not yet recovered completely from the long period of shrinkage which they had undergone subsequent to the maximum radius observed on July 17. Also, judging from the shapes of the curves, the radii of most, if not all, of the other groups of trees probably increased somewhat after the observations were terminated on October 17.

Data to be presented in detail elsewhere show that soils become exhausted of growth water to a considerable depth in the ponderosa pine zone, where the lowest group of pines was located; but in the arborvitae-hemlock and spruce-fir zones, in which all other groups of trees were situated, soils remain moist throughout the year. This seems to provide an explanation for the fact that, except in *Pinus*, the trees at their lower stations grew faster. Unless moisture is limiting, the lower the altitude, the greater the growth, on account of the higher temperature efficiency.

A feature of special interest in the data is the considerable difference in behavior observed between the two stands of *Pinus*, which were separated by only a little over 200 meters in altitude. As indicated above, the upper group of trees is situated where soils are favorably moist all summer. Undoubtedly, this difference in soil-moisture relations between the two habitats is far more important than the slight difference in temperature that is indicated by altitude. Therefore, it becomes apparent that the location of trees with respect to zones may be a much more significant guide to the type of radial behavior to be expected than are

strictly nonbiological data, such as elevation above sea-level.

The only previous study of radial behavior of trees growing at different altitudes in the same region appears to be that of FOWELLS (2), who studied *P. ponderosa* growing at altitudes ranging from 550 to 1830 meters in the Sierra Nevada Mountains of California. There is good agreement between the conclusions of that study and the present investigation, in that growth at low altitudes in California is likewise interrupted by a considerable period of shrinkage in late summer and that in April and May the rate of growth decreases upslope. Furthermore, the phenology of diametral growth is essentially identical in the two regions.

Periodic measurements of trees to determine their annual increment should always be made after the species has completed its seasonal growth. The present study reveals considerable difference among the species as to the earliest date when their growth can be considered essentially complete in northern Idaho. If the results of this single season's study are representative of average conditions— and there is no evidence to the contrary —the following dates are important.

Near their upper altitudinal limits the radii of *Larix* become relatively stable after mid-July, *Pinus* after August first, *Thuja* after mid-September, and *Abies* after mid-October. At its lower limits the radii of *Abies* remain fairly constant after mid-August. *Thuja*, at the lower station, exhibited a second growing season, which probably lasted beyond mid-October. With this tree, as well as with *Larix* and *Pinus*, it appears that reasonably significant measurements of annual increment at low altitude cannot be made until after the rainy season is well under way.

Summary

Radial changes were followed in 32 trees divided equally among four species of conifers growing on Thatuna Ridge in northern Idaho. Four trees in each species (*A. grandis*, *L. occidentalis*, *P. ponderosa*, and *T. plicata*) were located at relatively low altitude, and the other four at relatively high altitude. The period of observation extended from April 19 or May 15 until October 17, 1942.

In spring the most apparent effect of the vertically stratified climates on tree growth consisted of a well-marked retardation of cambial activity at high altitudes, which was probably due to the temperature gradient. In summer and early autumn the water-balance gradient exerted the most evident influence, as shown by differences in the amount of shrinkage. At low altitudes the trunks invariably shrank in late summer. At high altitudes shrinkage was either less pronounced or not evident.

The higher the altitude, the later the trees attained their maximal growth rates, and the later the effects of dry summer weather became evident. Pronounced differences in behavior were observed with as little as a 227-meter difference in elevation. This was correlated with differences in location with respect to vegetation zones and suggests a possible practical application of a knowledge of zonation.

The time when radial enlargement is completed varies widely among the four species and is strongly affected by altitude. In general, stability is attained at a later date at low altitude because the hydration of tissues formed in summer is deferred until after the rainy season begins in autumn.

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SOME EFFECTS OF ALTITUDE AND WATER SUPPLY ON THE COMPOSITION OF DERRIS ELLIPTICA

RUFUS H. MOORE[1]

Introduction

The effects of altitude and water supply on *Derris elliptica* (Wall.) Benth. have received only brief mention in the literature on this tropical legume. BUNTING and MILSUM[2] reported that plants flourished at 4750 feet above sea level at 4°28′ N. Lat. in Malaya but did not comment on the quality of root produced at this elevation. Growing *Derris* at more than 2300 feet in the Netherlands East Indies was not recommended (4). Although 80 or more inches annually of uniformly distributed rainfall have been considered a requirement for this species, supporting data are notably lacking. Records from Puerto Rico and Guatemala, presented herewith, show the influence of altitude and, to a limited degree, of water supply on the composition of derris root.[2]

Sources of samples

Single-plot tests conducted in several regions of Puerto Rico from 1940 to 1942 showed the effects of different combina-

[1] The author was formerly Plant Physiologist at the Federal Experiment Station, Mayagüez, Puerto Rico.

[2] All pH determinations and analyses for total chloroform extractives and rotenone were made by M. A. Jones, Chemist, Federal Experiment Station, Mayagüez, Puerto Rico. Mr. Jones and Gilda C. Vicente, Collaborating Agronomist, performed the carbohydrate analyses co-operatively.

tions of environment on the quality of derris root (10). Each plot contained from 225 to 275 transplants of the Sarawak Creeping variety, spaced 2 × 3 feet. A 12-8-5 commercial fertilizer was applied at the rate of 800 pounds per acre when these plants had resumed growth. The vines were allowed to trail naturally on the ground. The pH of the first and second foot of soil was determined with a Beckman pH meter when the vines had begun to form a soil cover. At harvest time all plants in a strip at least 6 feet wide were discarded to minimize border effects that had developed during the growth period. Only the roots of the 100 plants that had been inclosed by this border were analyzed. All samples in these regional trials and in the commercial plantings described in the next paragraph had some roots thicker than 1 cm.

In June, 1942, small commercial plantings of the Sarawak Creeping variety were made at three different elevations in Puerto Rico. In these fields exceptionally well-rooted plants were spaced 3 × 3 feet, and no fertilizer was used. Nineteen months later, samples were dug, each being a composite of part of the root systems of ten plants.

In August, 1940, the Department of Agriculture of Guatemala introduced from the Philippine Islands a variety of

Derris that closely resembles Sarawak Creeping. During the following spring, small lots of young plants were distributed to farmers on the Pacific watershed. Root samples were gathered in May, 1943, from plants supported on poles at elevations ranging from 910 to 4888 feet above sea level.[3] Portions of the root systems of from seven to ten plants were composited for each sample. Roots from

lengthwise to facilitate drying. The samples became snap-dry within 3–5 days when spread out in a thin layer in indirect light. This material was ground in a no. 2 Wiley mill until fine enough to pass through a 0.5-mm. sieve.

The powdered samples, which contained from 5.8 to 11.8 per cent of moisture, were stored in tightly stoppered bottles until analyzed for total

TABLE 1

ANALYSIS OF DERRIS ROOT GROWN UNDER DIFFERENT ENVIRONMENTAL
CONDITIONS IN PUERTO RICO

| PERIOD | SITE | FEET ABOVE SEA LEVEL | INCHES OF ANNUAL RAIN-FALL* | SOIL | | | | MONTHS AT HARVEST | PER CENT OF TOTAL CHLORO-FORM EX-TRAC-TIVES† | PER CENT OF PURE ROTE-NONE† | PER CENT OF CARBO-HY-DRATES† |
| | | | | Class | pH | | | | | | |
					First foot	Second foot					
1940 to 1942	Maricao	2400	98.9	Nipe clay	4.9	5.3	27	6.2	2.0	27.4
	Cidra	1400	79.1	Cialitos clay	4.7	4.7	24	13.3	4.3	19.0
	Utuado	380	73.4	Toa silty clay loam	6.1	5.4	28	19.6	6.0	......
	Sabána Grande	220	49.1	Jácana clay	6.8	7.3	24	16.4	4.5	10.9
	Vega Baja	215	70.2	Bayamón fine sandy loam	4.7	5.4	28	20.7	6.0	5.9
	Mayagüez	50	76.3	Toa silty loam	6.9	7.2	27	21.3	6.8	13.1
1942 to 1944	Orocovis	1970	68.9	Mucara silty clay loam	....		19	5.5	1.9	37.8
	Cidra	1410	89.7	Cialitos clay	acid		19	10.4	3.9	28.3
	Humacao	100	91.2	Humacao clay	acid		19	17.4	6.2	20.4

* The actual mean annual rainfall of the experimental period is given, except in the case of Cidra, for which incomplete 1940–1942 data made it necessary to use the mean rainfall over a period of years.
† Moisture-free basis.

higher altitudes were so much thinner than those from lower elevations that it seemed advisable to sort each sample into small and large roots. Small roots were less than 1 cm. in diameter, and large roots were 1 or more cm. thick.

Chemical methods

All roots were washed free of soil, and those thicker than 5 mm. were split

[3] The writer made these collections while on temporary assignment with the Office of Economic Warfare of the Foreign Economic Administration.

chloroform extractives (5), rotenone (1), and reserve carbohydrates. Sucrose was hydrolyzed with dilute HCl (1), and starch was digested with malt diastase followed by dilute HCl (1). The percentages of total sugars and of starch, estimated separately by the Lane-Eynon method (1), were added to give the values reported for reserve carbohydrates. "Hemicelluloses," determined on samples from the highest and lowest sites, varied from 6.1 to 9.3 per cent on a moisture-free basis. Since they showed

TABLE 2

ANALYSIS OF DERRIS ROOT HARVESTED IN 1943 AT SEVERAL ALTITUDES IN GUATEMALA

SITE	FEET ABOVE SEA LEVEL	INCHES OF ANNUAL RAINFALL*	MONTHS AT HARVEST	PER CENT OF TOTAL CHLOROFORM EXTRACTIVES†			PER CENT OF PURE ROTENONE†			PER CENT OF RESERVE CARBOHYDRATES†		
				Small roots	Large roots	All roots	Small roots	Large roots	All roots	Small roots	Large roots	All roots
Guatemala City..	4888	43.1	33	7.4	5.4	6.7	2.3	1.4	2.0	35.3	41.2	37.1
Finca Montevideo	3650	204.4	14	11.2	‡	11.2	3.5	‡	3.5	.....	.....	.....
Finca María Santísima.........	2050	99.0	24	17.7	11.7	16.1	7.0	4.4	6.3	19.8	36.2	24.2
Finca El Baúl....	1700	178.8	24	16.6	11.3	14.6	6.3	3.9	5.3	21.9	32.8	26.5
Finca Velásquez..	910	......	25	17.2	12.5	14.7	6.0	5.0	5.5	28.4	37.9	33.2

* The actual mean annual rainfall for the experimental period is given, except an 11-year average for Finca El Baúl. Data for Finca Velásquez was incomplete.

† Moisture-free basis.   ‡ No large roots in this collection.

no correlation with the known nutritional differences in the plants, complete analytical data on them have been omitted.

Results

Shoot development—luxuriant at lower sites with an adequate moisture supply—was observed to be progressively less vigorous as altitude increased or as water supply was restricted. In Puerto Rico, root nodules developed at all altitudes, but they were much larger at lower elevations. In Guatemala, no nodules were found on the roots collected at 4888 and 3650 feet, but they occurred in abundance on plants at elevations of 2050 feet or less.

Soil and climatological data and the chemical analyses of roots appear in tables 1, 2, and 3. Figures 1 and 2 correlate trends in the data.

Discussion

Both total chloroform extractives and rotenone are sometimes used to estimate the quality of derris root. Since variation in these two constituents followed similar trends, rotenone only will be used in this discussion as an index to root quality.

ALTITUDE AND TEMPERATURE

Figure 1 shows that, in general, the quality of derris root was inversely correlated with altitude and that roots were

TABLE 3

MEAN ANNUAL TEMPERATURES AT SEVERAL ELEVATIONS IN PUERTO RICO AND IN SOUTHWESTERN GUATEMALA

Country and station	Feet above sea level	Years	Mean annual temperature (°F.)
Puerto Rico: *			
Guineo Reservoir....	3000	15	66.7
Cayey.............	1400	41	73.0
Comerío Falls.......	500	37	76.8
Utuado.............	430	22	75.6
Manatí.............	250	42	77.0
Humacao...........	100	37	75.4
Mayagüez...........	80	43	77.2
San Juan...........	50	44	78.0
Guatemala: †			
Guatemala City.....	4888	13	64.9
Palín..............	3707	10	69.3
Mocá..............	2986	10	71.4
Escuintla..........	1115	10	77.4
Retalhuleu.........	787	10	79.0
Puerto San José......	0	12	82.8

* Data from U.S. Department of Commerce, Weather Bureau's *Climatological Data: West Indies and Caribbean Section,* 22, No. 13, 105. 1943.

† Data, secured through the co-operation of the Foreign Economic Administration's office in Guatemala City, was furnished by the courtesy of the National Observatory in Guatemala.

consistently higher in rotenone at greater altitudes in Guatemala than in Puerto Rico. Obviously, one or more factors associated with altitude caused these gradients of response in the derris plants.
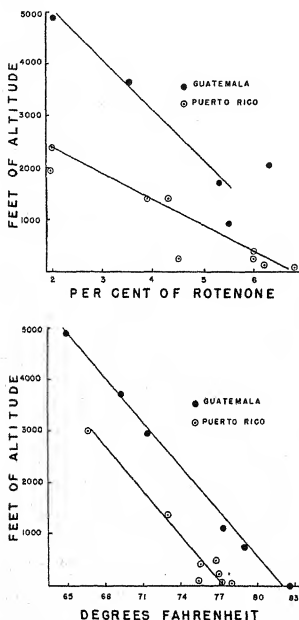
FIGS. 1–2.—Fig. 1, relationship between altitude and percentage of rotenone in derris root. Fig. 2, relationship between altitude and mean annual temperature.

Any correlation of rotenone content with intensity, duration, or quality of sunlight or with relative humidity cannot be considered because adequate meteorological records are not available. Inasmuch as mean temperature decreases as altitude increases, temperature could be a critical factor in the altitude effect. This possibility is indicated in figure 2, which shows that zones of the same mean annual temperature averaged from 1200 to 1300 feet higher in Guatemala than in Puerto Rico.

Geography accounts for higher temperatures at the same elevations in Guatemala than in Puerto Rico. The part of Guatemala from which roots were collected lies at approximately 14°40′ N. Lat., 3°35′, or about 247 miles nearer the equator than does Puerto Rico. Also, the Pacific watershed of Guatemala is protected from northerly winds by volcanic ranges and is part of a relatively large land mass on which temperatures are not moderated to the extent that they are in Puerto Rico, a small land mass in the direct path of the trade winds (7, 13). The inherently tropical adaptation of *Derris* shows clearly in its response to the temperature relation of altitude.

WATER SUPPLY

Although there was a close inverse correlation of root quality with elevation and temperature in the upper range of altitudes, divergent cases were found at lower elevations. Certain differences in water supply were associated with and probably caused these exceptions.

Rain was the only source of moisture for the derris plantings in Puerto Rico. Precipitation at the test sites can be roughly classified into two rainfall-distribution types. One type has a 4–5-month period of definite drought, and the other has appreciable rainfall in the so-called dry season. Maricao, Sábana Grande, and Mayagüez were characterized by seasons of definite and protracted drought. Orocovis, Cidra, Vega Baja, and Humacao received enough

rain to promote growth during the drier part of the year. Although Utuado did not fit readily into either type of rainfall distribution, its dry season was broken by fairly heavy rains. Vega Baja was outstanding in the uniformity of rainfall distribution, there having been only 3 months of the 28 in the crop cycle that had less than 2.6 inches. The rainfall-distribution patterns just described were too similar to exert an unmistakable effect on root quality.

Differences in total annual precipitation appear to have had no measurable effect on root quality at higher altitudes in Puerto Rico (see table 1), for roots of almost identical rotenone content were grown with 98.9 and 68.9 inches of rainfall at Maricao and Orocovis, respectively. But total annual precipitation was effective at lower elevations. For example, roots from Sábana Grande, where the mean annual rainfall was only 49.1 inches, were much lower in rotenone than those grown at similar low elevations receiving 70.2 and 73.4 inches of yearly precipitation.

Unlike Puerto Rico, the Pacific versant of Guatemala has only one rainfall-distribution pattern. The 6-month rainy season beginning in May is followed by 6 months of little or no rain (7). But, as one proceeds from the highlands toward the lowlands, mean annual precipitation decreases, and the dry season becomes intensified. It is during this part of the year that some fields are irrigated from the numerous mountain streams that transect the watershed.

No irrigation was used at altitudes of 4888, 3650, and 1700 feet. The derris root from these elevations showed a definite inverse correlation between rotenone and altitude. The extent to which irrigation supplemented rainfall had considerable bearing on the quality of root

secured from two of the sites within the range of altitudes ecologically favorable to *Derris*. Irrigated twice monthly during both dry seasons, the plants at 2050 feet had an almost uniform distribution of water throughout the year. As a result, they grew luxuriantly and produced the highest-quality root harvested in Guatemala. By contrast, the plants at 910 feet were irrigated only four times in the first dry season and three times in the second. Insufficient irrigation, especially in the second dry season, was probably the cause of the relatively low percentage of rotenone at this elevation.

OTHER FACTORS

Although most of the soils in Puerto Rico on which *Derris* was grown are classified as clay or clay loam, they were of good physical condition because of a definite crumb structure. Differences in the pH of these soils had no apparent effect on the quality of derris root (see table 1). Volcanic in origin, the Guatemalan soils are rich, deep, and well drained. Some of those examined at higher altitudes have a considerable admixture of fine cinders, but those at lower elevations have the texture of silty loam or fine sandy loam. On the whole, there was more variation among the Puerto Rican soils than among the Guatemalan soils. The data presented are insufficient to indicate whether soil type in either country appreciably affected the quality of derris root.

Although the plants grown in both countries had the characteristics of the Sarawak Creeping variety, they were sufficiently different from each other to be two distinct intravarietal selections. Apparently, they responded differently to the decline in temperature with altitude. Figure 2 shows that the temperature gradients in both countries were ap-

proximately equal to the normal lapse rate of $1°$ F. fall in temperature with each 300 feet of vertical ascent (3). However, figure 1 shows that, as elevation increased, the quality of derris root was not lowered so rapidly in Guatemala as in Puerto Rico. This might indicate that the derris selection in Guatemala is better adapted to ecologically higher altitudes than the Sarawak Creeping selection of Puerto Rico.

It is possible that the methods of vine management employed in the two countries lessened the observed differences in the quality of root produced at the same elevation. Guatemalan *Derris* was trained onto poles, while Puerto Rican *Derris* was allowed to trail over the soil. As the use of vine supports lowers the percentage of rotenone in the roots (9), the use of poles in Guatemala probably did not favor the production of roots of maximum quality.

Harvest age affects the quality of derris root grown at a favorable altitude. The extent to which the rotenone content can be expected to change with age was demonstrated in the Sarawak Creeping variety at Mayagüez, where the percentage of rotenone rose from 5.0 at 18 months to 5.7 at 24 months and then declined to 5.2 at 27 months (10). Roots produced at low and medium elevations in Puerto Rico exemplified part of this normal change in analytical values. Roots harvested near Cidra when 19 and 24 months old had 3.9 and 4.3 per cent of rotenone, respectively. Similarly, 19-month-old roots from Humacao had 6.2 per cent of rotenone in comparison to 6.8 per cent in 27-month-old roots from Mayagüez. On the other hand, the few data that are available indicate that the age effect was practically obscured at higher altitudes. In Puerto Rico, the difference between 1.9 and 2.0 per cent of rotenone in roots 19 and 27 months old, respectively, falls within the limits of analytical error. In Guatemala the minimum difference of 43 per cent between 3.5 and 2.0 per cent of rotenone in roots 14 and 33 months old is much greater than the normal variation in root quality in this range of ages.

ROTENONE-CARBOHYDRATE RELATIONSHIPS

A pronounced restriction of the water supply (6) or a lowering of temperature (12), either of which increases the carbohydrate reserves in other species of plants, causes a similar response in *Derris* (tables 1, 2, and 3). Even though carbohydrates are not the basis for evaluation of derris root as an insecticide, they are of special interest because they normally have a reciprocal relationship to rotenone.

Rapid growth of derris plants causes increased elaboration of rotenone and less storage of carbohydrate reserves, but slow growth is accompanied by relatively slow deposition of rotenone and comparatively rapid accumulation of carbohydrates (11). Rotenone and carbohydrate reserves maintain this inverse relationship when the nutritional level of the plant does not fluctuate excessively. This normal effect of growth vigor on rotenone-carbohydrate relationships is clearly evident in the Puerto Rican harvests of 1944. It was observed that shoot growth of the plantings at Orocovis, Cidra, and Humacao was of practically the same order as the rotenone percentages in the roots, but the carbohydrate percentages were in the reverse order. The same rotenone-carbohydrate relationship also appears in the 1942 Puerto Rican data for Maricao, Cidra, and Mayagüez and in the 1943 data for Guatemala City and Finca El Baúl.

Because reserve carbohydrates, but not rotenone, can be utilized in normal metabolic processes of the derris plant (8), the rotenone-carbohydrate relationships may not always be those just described. In general, the extent of carbohydrate accumulation in Puerto Rican plants varied with the season. The 1944 harvests were made after the dry season had begun, when growth had been checked and carbohydrates had accumulated to relatively high percentages. On the other hand, the 1942 harvests were made during the rainy season or shortly after the onset of the dry season, when rapid growth was utilizing carbohydrate reserves. Sudden increases in moisture several days prior to the Vega Baja and Sábana Grande harvests initiated growth flushes that reduced carbohydrate reserves to exceptionally low values.

Inasmuch as the root samples from Guatemala were gathered at the end of the dry season, all were high in carbohydrates. Nevertheless, the effect of water supply was reflected in the concentration of these reserves. The frequent irrigation of plants grown at 2050 feet produced vigorous growth that prevented carbohydrates from accumulating to the extent that they would have if rainfall had been the only source of water at this elevation. By contrast, the less frequent application of water to plants at 910 feet, in conjunction with the more intense dry season at this lower elevation, allowed carbohydrates to increase to a value higher than would be indicated by their rotenone content.

Tip-burned leaves were evidence that these plants had suffered from serious water shortage during the last few months prior to harvest.

Summary

1. *Derris elliptica* was grown in Puerto Rico at elevations ranging from 50 to 2400 feet above sea level and in Guatemala at altitudes ranging from 910 to 4888 feet.

2. The temperature factor of altitude correlated positively with rotenone and inversely with reserve carbohydrates in derris roots.

3. At elevations favorable to growth of derris plants, the accumulation of rotenone was influenced by major variations in water supply, but reserve carbohydrates were altered by relatively minor variations in available moisture.

4. The varietal selection of *Derris* grown in Guatemala appeared to be more adapted to higher elevations, in an ecological sense, than the Sarawak Creeping variety grown in Puerto Rico.

5. The normal effect of age on the percentage of rotenone was apparently obscured at high altitudes.

6. Soil pH, ranging from values of 4.7 to approximately neutral, had no measurable effect on rotenone or carbohydrate reserves.

7. At favorable altitudes, derris plants flourished in soils having good physical structure and belonging to several distinct soil types.

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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

This issue of the BOTANICAL GAZETTE contains eighteen papers originating at Camp Detrick, Frederick, Maryland. The work reported therein was carried out as a part of the activities of the Special Projects Division, Chemical Warfare Service, in 1944 and 1945. Earlier publication of this material was incompatible with wartime security policies determined by the War Department.

This work was developed from the base laid by many workers on growth-regulatory and formative effects produced by hetero-auxin, naphthalene acetic acid, and similar or related compounds. As a result of various experiments in the late thirties and early forties, the high activity of certain of these synthetic growth-regulating substances was recognized. This led to the suggestion by E. J. Kraus that they might find use for herbicidal purposes and thence to the initiation of tests in which inhibitory potency was established.

Other laboratories and groups were also involved in the project on a contract basis. J. W. Mitchell, of the Bureau of Plant Industry, United States Department of Agriculture, Beltsville, Maryland, participated from its initiation and, together with E. J. Kraus, carried out in February, 1944, at the University of Chicago, certain key experiments that provided unequivocal proof of the herbicidal activity of a few substituted phenoxyacetic and benzoic acids. Melvin S. Newman, Department of Chemistry, Ohio State University, was responsible for the synthesis of a substantial number of compounds for test. All these individuals conformed to the security requirements of the project and consequently withheld from publication their findings. In some instances, also, publication of the results of other related physiological work upon which they were currently engaged was delayed. The subject of the synthetic plant growth-regulating substances and, in particular, their application for herbicidal purposes will therefore not be represented in the scientific literature in wholly correct chronology. In this connection it must be borne in mind also that important developments in this field took place entirely independently in Great Britain.—A. G. NORMAN.

CAMP DETRICK
FREDERICK, MARYLAND

NEW GROWTH-REGULATING COMPOUNDS. I. SUMMARY OF GROWTH-INHIBITORY ACTIVITIES OF SOME ORGANIC COMPOUNDS AS DETERMINED BY THREE TESTS[1]

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Introduction

The systematic study of organic compounds of known structure in relation to physiological effects on plants may be said to have been initiated in 1931, when F. KÖGL and his collaborators—HAAGEN SMIT, ERXLEBEN, TONNIS, and KOSTERMANS—began investigations on the identification of the *Wuchsstoff* (29). In 1935, KÖGL (28) published the assigned structure of auxentriolic acid (auxin-a) and auxenolonic acid (auxin-b) and noted the similarity between the physiological activities of hetero-auxin (beta-indoleacetic acid) and the auxins. This observation was confirmed by THIMANN and KOEPFLI (43).

KÖGL (28) and KÖGL and KOSTER-

MANS (30) used the *Avena* coleoptile test for the determination of regulating activity of some thirty-four substances, including derivatives of the auxins, heteroauxin, 2- and 3-indolecarboxylic acid, beta-(3-indole)-propionic acid, 3-indolepyruvic acid, and beta-(3-indole)-alpha-amino-propionic acid. HAAGEN SMIT and WENT (14) investigated three different methods for testing physiological activity and studied thirty-three substances, mostly indole derivatives, substituted phenylacetic and cinnamic acids, and some alkyl carboxylic acids. THIMANN (41) presented an analysis of the activity of 3-indoleacetic acid, and the analogous indene and coumaryl (benzofuran) derivatives on plant tissues. Various indole-substituted aliphatic acids were tested by BAUGUESS (1) for root initiation, stem bending, and bud inhibition of tomatoes, marigolds, and stocks. HITCHCOCK (19, 20) reported the effects of indoleacetic, phenylacetic, and naphthaleneacetic acids and several of their homologues on leaves of the tobacco plant. ZIMMERMAN and WILCOXON (59) tested alpha- and beta-naphthaleneacetic acid, 5-acenaphtheneacetic acid, gamma-indolebutyric acid, phenylacetic acid, 3-indoleacetic acid, fluoreneacetic acid, anthraceneacetic acid, alpha-naphthaleneacetonitrile, gamma-(2-carboxy-3-indole)-butyric acid, and indolepropionic acid on sweet peas and other plants.

The root-initiating activity of a variety of indoleacetic and similar compounds was tested by ZIMMERMAN and HITCH-

cock (47). This was extended by HITCH-COCK and ZIMMERMAN (21) to include studies of the absorption and movement of such synthetic growth substances from soil, as judged by the responses produced in the aerial parts of the plants, and of the rooting responses of cuttings (22). Skatole (3-methylindole) was tested as a growth-regulating substance by GLOVER (12). Alkyl esters of alpha-naphthaleneacetic, phenylacetic, indoleacetic, indolepropionic, and indolebutyric acids were compared in activity on tomato, sunflower, and other plants by ZIMMERMAN, HITCHCOCK, and WILCOXON (57).

GAVAUDAN, GAVAUDAN, and POMRIA-SKINSKY-KOBOZIEFF (11) in 1937 published observations on the effects of colchicine on onion roots, and HAVAS (17, 18) the responses of wheat seedlings to the same compound. In the same year ZIMMERMAN (46) reviewed the responses of plants to hormone-like substances and, with HITCHCOCK (48), tested the relative effectiveness of inorganic and organic salts of alpha-naphthaleneacetic, beta-indoleacetic, beta-indolepropionic, and gamma-indolebutyric acid on tomato and other plants, bending responses, root initiation, retardation of growth, and swelling of treated tissues all being used in the evaluation. THIMANN (42) studied the nature of the inhibitions caused by the application of auxin to *Pisum* seedlings. GARDNER and MARTH (10) described the development of parthenocarpic fruits following spray treatment with various growth-promoting substances in very low concentrations.

The histological reactions produced in bean plants by some growth-regulating compounds were investigated by HAM-NER and KRAUS (15). BEAL (3) described the bud development in *Lilium harrisii* caused by treatment with indoleacetic

acid and later extended this to a study of the histological responses produced in three species of *Lilium* by treatment with the same substance.

ZIMMERMAN and HITCHCOCK (49) investigated the tropic responses of leafy plants treated with growth-regulating substances and later (50) the combined effects of light and gravity on the responses of plants so treated. HITCHCOCK and ZIMMERMAN (23) described the use of tomato and other cuttings in the determination and evaluation of physiological activity. ZIMMERMAN, HITCH-COCK, and WILCOXON (58) gave the results of tests of twenty-nine substances, including alpha- and beta-naphthoxy-acetic acids applied as vapors or in solution to corn, tomato, pea, and other plants. Nastic curvatures, tropic responses, swellings, root initiation, retardation or inhibition, and partheno-carpy were all described. BAUSOR (2) reported the effects of beta-naphthoxy-acetic acid on tomato plants, and MUL-LISON (34) the responses of bean plants to tetrahydrofurfural butyrate. BLOCH (8) discussed the use of synthetic growth-regulating substances on plants.

The number of naphthoxyacetic acid derivatives demonstrated to have plant growth-regulatory activity and to produce formative growth effects was considerably extended by ZIMMERMAN (44) and by ZIMMERMAN and HITCHCOCK (51, 52). In 1942 the same authors (53, 54) reported the testing of halogen, nitro-, and amino-substituted phenoxyacetic and benzoic acids on a variety of plants with particular reference to epinasty, modification of organs, initiation of adventitious roots, inhibition of buds, formative influences, and parthenocarpic fruit setting. By this time a sufficient number of substituted phenoxy compounds had been studied to permit in-

vestigation of the relationships between chemical structure and root-inducing activity (24, 25, 26). ZIMMERMAN (45) further reviewed the information available on the formative influence of growth-regulating substances on plants. MARTH (32) described experiments on the effects of a number of naphthalene and indole derivatives on shoot development of roses.

In 1944 various workers (5, 16, 33) independently suggested in print the use of synthetic growth-regulating substances as selective herbicides. Not in every case, however, was proof of herbicidal or phytotoxic action given, nor was selective activity demonstrated. MITCHELL and HAMNER (33) described the use of certain polyethylene glycols as carriers in aiding in the preparation of aqueous solutions of some of the more insoluble growth-regulating substances. The results of applications of this type in thirty-one substances of this type in the aerosol form were reported by ZIMMERMAN and HITCHCOCK (55); and later the same workers, with HARVILL (56), tested fourteen substituted xylenoxy acids for activity comparable to the phenoxy acids.

SLADE, TEMPLEMAN, and SEXTON (37) in 1945 published a list of thirty-two aryloxyacetic acid derivatives that had been tested for herbicidal activity, some, it was stated, as early as 1941. They drew attention particularly to the high promise of 2-methyl-4-chlorophenoxyacetic acid for this purpose. BLACKMAN (7) compared the behavior of this acid with that of 2,4-dichlorophenoxyacetic acid as a selective herbicide, and NUTMAN, THORNTON, and QUASTEL (35) described experiments on these two substances in soil.

In the same year SYNERHOLM and ZIMMERMAN (38) prepared 2-chloro-3,5-diiodo- and 2-chloro-3,5-dibromobenzoic acids and investigated the effects produced by them on tomato plants and also (39) reported tests of forty-six aryloxy-alkyl carboxylic acids for plant growth-regulating activity, using as criteria the threshold concentrations in lanolin causing cell elongation and formative effects. BEAL (6) continued histological studies of tissues of plants treated with certain phenoxyacetic compounds.

Plant growth-regulating activity of a somewhat different character has been found to be a property of certain compounds of the urethane series. The earliest tests reported in the literature appear to be those of FRIESEN (9) in 1929, who investigated the action of phenyl and ethyl urethane on oat and wheat seedlings. LEFÈVRE (31) commented on the similarity of the reactions caused in some plants by colchicine and phenyl urethane. SIMONET and GUINOCHET (36) and GUINOCHET (13) also noted that phenyl urethane and certain other compounds caused colchicine-like changes in wheat and wheat seedling. A number of urethanes were described by TEMPLEMAN and SEXTON (40) in 1945 as possessing some activity in causing inhibition of cereal seedlings. Isopropylphenylcarbamate was said to be the most active of these relatively insoluble compounds.

Early in 1944 a project centered at Camp Detrick, Maryland, was initated for the study of inhibitory activities of synthetic plant growth-regulating substances. By August, 1945, almost eleven hundred substances had been synthesized and submitted to test in the laboratory and greenhouse. The more promising substances were further studied in greenhouse and field on a wide range of plant species. In this paper are summarized the experimental data obtained in screening these compounds by three tests employed routinely. All compounds are

included, whether proved to have activity or not.

Methods

Considerable diversity exists between methods used by investigators for the evaluation of compounds as plant growth-regulating substances (9, 14, 19, 20, 21, 22, 23, 27, 33, 39, 41, 48, 55, 58), but none of these was satisfactorily applicable to the objectives of this project. In most cases the results of previous tests were not capable of quantitative expression in such a manner as to permit comparison of activities of groups of substances tested at different times. Perhaps most closely approaching the requirements was the device of establishment of threshold concentrations of any compound necessary to cause the appearance of some readily recognizable plant reaction. This has been principally used by HITCHCOCK and ZIMMERMAN in their work. Since, however, gross inhibition of growth was considered to be the most likely manifestation of phytocidal activity in this class of compound, the effects of which are telemorphic and in great contrast to those produced by contact herbicides, all tests were based on weight or length changes following treatment with a uniform quantity of the compound under test. In order that seasonal or environmental effects should be minimized, a common reference material was employed in all test groups, and the results of any test were expressed as a percentage of the inhibition produced concurrently by the reference material. The reference material used in each of the three tests reported in this communication was 2,4-dichlorophenoxyacetic acid, commercial grade purified through the ammonium or alkali metal salt by several recrystallizations from aqueous and alcoholic solutions.

The primary test involved the determination of inhibition of elongation of the primary root of germinating corn. This method in detail follows.

TEST A. CORN-GERMINATION TEST

Corn grains of the variety of Silver King (Wisconsin No. 7) were germinated at a constant temperature of 27° C. on filter paper in covered 6-inch Petri dishes, to which were added 20 ml. of aqueous solution[2] of the compound to be tested, at a concentration of 10 p.p.m. Twenty-five seeds were used in each dish, and three dishes were used with each compound. After 4 days of growth, the length of the primary root was measured. Inhibition of growth was determined by subtracting the average length of the primary root of the treated seeds from that of the control or untreated seeds. Inhibition of growth caused by the application of 2,4-dichlorophenoxyacetic acid was arbitrarily designated as 100%, and inhibition of root growth resulting from all other compounds was compared in terms of this percentage. Treatments with 2,4-dichlorophenoxyacetic acid were included in each group of tests.

In addition, two other methods were employed for the evaluation of the plant growth-regulating activity of chemical compounds and are reported for purposes of additional information. The methods were based on the application of a single drop of solution of the compound under test to young kidney-bean plants, under greenhouse conditions, and measurement of the effect of the treatment upon the subsequent vegetative growth. In one test the compound was applied in aqueous solution and in the other in oil, a

[2] In some cases it was found necessary to add small amounts of extremely dilute solutions of ammonium hydroxide or of acid to effect complete solubility of the compound for this test.

co-solvent being used where necessary. The details of these tests follow.

TEST B. THE KIDNEY-BEAN SINGLE-DROPLET WATER TEST

Kidney-bean seeds were planted in 4-inch pots containing approximately 1 pound of composted soil. Seven to 10 days after planting, the seedlings were approximately 4 inches in height and had developed primary leaves that were about $1\frac{1}{2}$ inches in width. The pots were then thinned to one plant per pot, nine plants being treated with each test substance. Each plant was treated with 0.02 ml. of an aqueous solution containing 200 p.p.m. (4γ) of the compound. This solution included sufficient Carbowax No. 1500 to furnish a 0.5% solution of the wax. The treatment was applied to the upper surface of one of the primary leaves at a point along the midrib and approximately $\frac{1}{8}$ inch from the point of attachment of the blade and petiole, a $\frac{1}{10}$-ml. pipette graduated in hundredths being used to apply the droplet. The fresh weight of that portion of each plant above the second node was obtained the 10th day after treatment. Inhibition of growth due to the use of 2,4-dichlorophenoxyacetic acid was designated as 100%, and the effect of other compounds was compared on this basis. Untreated controls were included in each series of tests.

TEST C. THE KIDNEY-BEAN SINGLE-DROPLET OIL TEST

Essentially the same procedure was employed in the kidney-bean single-droplet oil test as in the kidney-bean single-droplet water test. The differences were (1) there were two kidney-bean plants per 4-inch pot (three pots per series); (2) the treatment was made at the time the primary leaves were fully expanded and the second internode was approximately 1 inch long (at which time the plant was approximately 2 weeks old); and (3) 0.01 ml. of solution was used in the treatment. The concentration of the solution was such that a drop (0.01 ml.) contained 5γ in oil of the compound to be tested. When tributylphosphate was used as a co-solvent, as was the case with all compounds that were not directly oil-soluble or oil-miscible, the concentration of this substance was usually 0.2%. Harvest of fresh weights was effected in 10 days, and all calculations were carried out as in the kidney-bean single-droplet water test.

Discussion and Interpretation of Tests

In considering the results of the tests listed, consideration must be given to the reasons for establishment of each of the tests and the limitations inherent in them. Close numerical agreement between the results of the three tests is not necessarily to be expected, not only because different levels of growth activity may be manifest under the test conditions prevailing but also because in some cases the physical properties of certain of the compounds were such as to make difficult or impossible their testing at the desired concentration. For example, many compounds synthesized, though not completely water-insoluble, had such a low solubility that it was not found feasible to prepare with confidence for Test A a solution containing 10 p.p.m., which necessarily has to be done by diluting from a more concentrated solution. Many of these relatively insoluble compounds, however, could be handled in Carbowax to give the necessary 200-p.p.m. solution for Test B. Compounds of the classes of acid halides and acid anhydrides included in these tests may react with water to give the correspond-

ing organic acid. In such cases and in those instances when ammonium hydroxide or hydrochloric acid was used to effect solution in water, the possibility of some chemical change occurring must be taken into consideration.

All tests were carried out with adequate replication, and the results reported are means of the treatments. It was possible to examine statistically the level of significance within any one group of compounds tested concurrently. This, however, has not been done for the whole group, because the position of any one compound with respect to another is not absolute but is dependent to some extent on environmental and seasonal conditions. The degree of inhibition produced by the reference compound in Tests B and C at different times of year was not wholly identical and was affected by such factors as rate of growth of the test material. Test A was undoubtedly the most reproducible, and for that reason and because of its simplicity it formed the primary basis for detection of inhibitory activity. The errors introduced by variation in the replicates were, on the whole, much smaller when the activities of the compound under test were high. Minimum differences necessary for significance are therefore generally greater within Groups IV-A or IV-C than in Group I.

It is to be noted that all tests contain a quantitative factor and that therefore the absence of inhibitory activity in the test is not to be taken as proof of absence of growth-regulating properties. Many of the compounds in Group IV-C, though manifesting little or no activity at the levels tested, might well prove active at higher concentrations. Compounds of those groups possessing significant activity by one or more tests described may be used as herbicides. Others may have

possessed growth-regulatory effects not manifest primarily in gross inhibition. None of these tests was designed to reveal formative effects quantitatively, though abnormalities in the growth habit of the plants were frequently observed in Tests B and C. Any consideration of relationships between structure and physiological activity should be conducted with these facts in mind.

In Test A it was sometimes found that the mean length of the roots of treated seeds was greater than that of the untreated control. Such results are recorded as a negative value for inhibition, expressed, as in the case of positive values, as a percentage of the inhibition effected by the reference material. Small negative values are probably not significant and are due to the normal variability of biological material. Larger figures, however, do indicate over-all growth stimulation, indirectly expressed by the system followed here. It would be desirable that such compounds be investigated further by more appropriate methods designed specifically to test for cell or tissue elongation or growth stimulation.

In Test B the value "o" was recorded in all cases in which the mean fresh weight of the new growth was equal to, or greater than, that of the untreated controls. Negative values, however, were recorded in Test C when greater growth was made by treated plants than by controls. Values greater than 100 occurred in a number of instances. By this it must be understood that the mean fresh weight of the new growth of treated plants exceeded that of the controls by an amount more than equal to the inhibition produced by the reference compound. In such cases the test compound did not necessarily produce enhanced normal growth; frequently, there was tissue proliferation and gall production

at the growing-point. In general, inhibitory effectiveness on kidney-bean plants was accompanied by tissue deformation and abnormal proliferation at lower rates of application.

When the results of the tests are viewed as a whole, the corn-germination test (Test A) proved to be reliable in separating those compounds that possess high inhibitory activity for most broadleaved plants from those with little or no activity at the same concentration. It is paradoxical that activity on established monocotyledonous plants, and on the Gramineae in particular, cannot be predicted from this test. The aqueous single-droplet test on the kidney bean (Test B) proved also to be a reliable index of probable inhibiting or herbicidal activity of compounds applied in the form of aqueous sprays to many broadleaved species. In general, the results of this test agreed fairly satisfactorily with those of Test A, though the spread was somewhat greater and some discrepancies were found. These were almost all in the direction of a lower activity in Test B than in Test A. Examination of Group IV-A reveals very few compounds substantially more active by Test B than by Test A.

The oil single-droplet test (Test C) was established in order to evaluate oil-miscible compounds having little or no aqueous solubility and can be used as a basis for selection of inhibitory compounds for incorporating in oil sprays for herbicidal purposes. By the expedient of employing tributylphosphate as a co-solvent, it was found possible to include in this test many compounds not directly oil-soluble or oil-miscible. The reference material—2,4-dichlorophenoxyacetic acid—could itself be introduced into oil only in this way. Variation between replicates in this test was always greater than in Test A or Test B. However, the results obtained were satisfactory in separating active inhibitors from those of low or little activity. Subsequent spray tests usually confirmed predictions as to herbicidal activity on broadleaved plants made from the results of Test C. Many of the numerical values obtained in this test are substantially higher than those recorded for Tests A and B. This is explained by the fact that the reference material—2,4-dichlorophenoxyacetic acid, which was a fortunate choice for Tests A and B—stands somewhat lower in activity in oil with respect to many of the other compounds tested than it does in aqueous solution, even though its absolute activity when distributed in oil is certainly no lower than when in water. Some compounds, though fully water-soluble, were considerably more active in oil than in water.

Results

The results obtained in testing this series of compounds are given in Tables 1–6. The compounds are classified into groups according to the percentage of activity of 2,4-dichlorophenoxyacetic acid by the corn-germination test (Test A): Group I, 80% or higher; Group II, 50–79%, inclusive; Group III, 30–49%, inclusive; Group IV-A, 29% or lower by Test A, but 50% or higher by Tests B and/or C; Group IV-B, not sufficiently soluble for Test A, but 50% or higher by Test B and/or C; Group IV-C, other compounds tested. The compounds are listed alphabetically in each group.

Summary

1. The literature relating to the testing of organic compounds for growth-regulating activity on plants has been reviewed chronologically.

2. Three methods have been devised, whereby inhibitory activity at high dilu-

tion can be determined and evaluated. All depend on elongation or weight changes subsequent to treatment. The activity of the compounds under test are expressed as a percentage of the inhibition brought about concurrently by 2,4-dichlorophenoxyacetic acid as a common reference material. The plant materials used are germinating corn and young kidney-bean plants.

3. One thousand and sixty organic compounds, almost all synthesized expressly for this work, were submitted to test by the three methods. The data obtained have been assembled in groups on the basis of activity in reducing elongation of the primary root of germinating corn as compared with the reference material.

4. The significance and interpretation of the tests are discussed. Compounds showing high activity are indicated as promising for use as herbicides.

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TABLE 1

ACTIVITIES OF COMPOUNDS: GROUP I

Compounds possessing 80% or more of the activity of 2,4-dichlorophenoxyacetic acid in the corn-germination test (Test A), listed in alphabetical order. The results of the kidney-bean single-droplet water test (Test B) and the kidney-bean single-droplet oil test (Test C) are similarly expressed as the percentage of the activity of 2,4-dichlorophenoxyacetic acid in the same tests.

COMPOUND	TEST A	TEST B	TEST C
2-bromo-4-chlorophenoxyacetic acid*.	101	69	13†
butyl 2,4,5-trichlorophenoxyacetate.	84	149	128†
2-chloro-4-bromophenoxyacetic acid*.	80	84	52†
4-chlorocinnamic acid, ammonium salt*	90	23‡	....
4-chlorophenoxyacetamide.	84	174	ns§
3-chlorophenoxyacetic acid*	85	0	−158†
4-chlorophenoxyacetic acid*	85	107	162†
2,4-dichlorophenoxyacetamide.	94	57	ns§
2-(2',4'-dichlorophenoxyacetamido)-1-butanol	81	16‡	ns§
4-(2',4'-dichlorophenoxyacetamido)-2,5-dichlorobenzenesulphonic acid, sodium salt.	100	48	69†
2-(2',4'-dichlorophenoxyacetamido)-2-ethyl-1,3-propanediol	88∥	96‡	12†
2-(2',4'-dichlorophenoxyacetamido)-2-(hydroxymethyl)-1,3-propanediol	93	55	ns§
2-(2',4'-dichlorophenoxyacetamido)-2-methyl-1,3-propanediol.	8	102	12†
2-(2',4'-dichlorophenoxyacetamido)-naphthalene-1-sulphonic acid.	91	36	128†
1-(2',4'-dichlorophenoxyacetamido)-naphthalene-8-sulphonic acid.	92∥	29	ns§
1-(2',4'-dichlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid.	95	65	ns§
3,4-dichlorophenoxyacetic acid*.	89	55	107†
2,5-dichlorophenoxyacetic acid*.	96	8	−52†
2,4-dichlorophenoxyacetic anhydride.	102	13	97†
2,4-dichlorophenoxyacetic 4'-sulphoanilide.	104	0	30
2,4-dichlorophenoxyacetohydroxamic acid.	95	65	ns§
2,4-dichlorophenoxyacetyl chloride.	107	58‡	137†
2,4-dichlorophenoxyacetyl guanidine.	101	89	ns§
N-(2,4-dichlorophenoxyacetyl) urea.	99	117	ns§
alpha-(2,4-dichlorophenoxy)-butyric acid*.	96	7	92†
beta-(diethylamino)-ethyl 2,4-dichlorophenoxyacetate*.	100	84	90†
beta-(diethylamino)-ethyl 2,4,5-trichlorophenoxyacetate*.	104	53	200†
2,2-dimethyl-1,3-dioxolane-4-methanyl-2'-methyl-4'-chlorophenoxyacetate*.	100	3‡	−22
1,4-di-(2',4',5'-trichlorophenoxyacetamido)-benzene.	100	102	ns§
,3-di-(2',4',5'-trichlorophenoxyacetamido)-benzene.	99	89	ns§
ethyl 2,4-dichlorophenoxyacetate*.	96	100	66
ethyl 2-methyl-4-chlorophenoxyacetate*.	99∥	·1	118
ethyl alpha-(2-methyl-4-chlorophenoxy)-heptoate*.	85∥	0‡	−50
beta-hydroxyethyl 2,4-dichlorophenoxyacetate*.	94¶	117	93
2 iodo-4-chlorophenoxyacetic acid*.	88¶	0	14†
2-methyl-4-bromophenoxyacetic acid*.	92	0	−25†
2-methyl-4-chlorophenoxyacetamide.	103	24	ns†
N-methyl-4-chlorophenoxyacetamide.	80∥	50‡	34†
4-(2'-methyl-4'-chlorophenoxyacetamido)-benzenesulphonic acid	85∥	48	57†
2-(2'-methyl-4'-chlorophenoxyacetamido)-naphthalene-6,8-disulphonic acid.	94∥	3	99†
2-(2'-methyl-4'-chlorophenoxyacetamido)-naphthalene-1-sulphonic acid	81	0	ns§
1-(2'-methyl-4'-chlorophenoxyacetamido)-naphthalene-8-sulphonic acid	92∥	0	ns§

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.
∥ Tested as NH4 salt after addition of sufficient NH4OH to effect solution.
¶ Tested after addition of sufficient HCl to effect solution.

TABLE 1—*Continued*

Compound	Test		
	A	B	C
2-(2'-methyl-4'-chlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid	83	o	ns§
2-methyl-4-chlorophenoxyacetic acid*	114	48	49†
2-methyl-6-chlorophenoxyacetic acid	81‖	o	ns§
2-methyl-4-chlorophenoxyacetic anhydride	101	o	58†
2-methyl-4-chlorophenoxyacetyl chloride	95	34‡	59†
2-methyl-4-fluorophenoxyacetic acid*	107	100	123†
N-methyl-2,4,5-trichlorophenoxyacetamide	103	70	ns§
2-nitro-2-methylpropyl 2',4'-dichlorophenoxyacetate*	99	103	82†
2-nitro-2-methylpropyl 2'-methyl-4'-chlorophenoxyacetate*	84¶	32‡	132†
phenyl chloroacetate	102	39	20†
phenyl 2-methyl-4-chlorophenoxyacetate	83	62‡	−36†
isopropyl 2-methyl-4-chlorophenoxyacetate*	99‖	o‡	155
2-(2',4',5'-trichlorophenoxyacetamido-2-(hydroxymethyl)-1,3-propanediol	94	247	ns§
2,4,5-trichlorophenoxyacetic N-di-(2'-hydroxyethyl)-amide	81¶	o‡	120†
2,4,5-trichlorophenoxyacetic piperidide	99	56	ns§
2,4,5-trichlorophenoxyaceto-3'-chloroanilide	81	68	226†
2,4,5-trichlorophenoxyaceto-2',4'-dimethylanilide	88	18	230†
2,4,5-trichlorophenoxyaceto-4'-ethoxyanilide	95¶	49	ns§
2,4,5-trichlorophenoxyaceto-4'-methylanilide	94	32	143†
2,4,5-trichlorophenoxyaceto-2',4',6'-trichloroanilide	102‖	68	217†
3-(trifluoromethyl)-phenyacetic acid*	94	47	−134†
N-(tris-[hydroxymethyl]-methyl)-N-(beta-hydroxy, gamma-(tris-[hydroxymethyl]-methylamino)-propyl 2,4-dichlorophenoxyacetamide, hydrochloride	86	o	14†

TABLE 2

ACTIVITIES OF COMPOUNDS: GROUP II

Compounds possessing 50–79% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A), listed in alphabetical order. Results of kidney-bean single-droplet water test (Test B) and kidney-bean single-droplet oil test (Test C) similarly expressed as percentage of activity of 2,4-dichlorophenoxyacetic acid in same tests.

COMPOUND	TEST		
	A	B	C
beta-aminoethanol bis-(4-chlorophenoxyacetate)*..................	65‖	25‡	114†
4-bromophenoxyacetic acid*.................................	50	70	50†
O-(2-carboxymethoxy-3-methyl-5-bromobenzoyl)-glycolic acid......	65	64‡	5†
O-(2-carboxymethoxy-3-methyl-5-nitrobenzoyl)-glycolic acid........	57‖	20	15†
monocapryl acid orthophosphate..............................	69‖	....	....
2-chloro-4-tertiary-butylphenoxyacetic acid*...................	52	50	0†
2-chloro-4-iodophenoxyacetic acid*...........................	70	7‡	20†
alpha-chloronaphthyl-acetic acid (mixture), ammonium salt*........	54		
2-(4'-chlorophenoxyacetamido)-naphthalene-1-sulphonic acid.......	62	2	ns§
1-(4'-chlorophenoxyacetamido)-naphthalene-4-sulphonic acid........	59	28	ns§
1-(4'-chlorophenoxyacetamido)-naphthalene-8-sulphonic acid........	65	46	ns§
1-(4'-chlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid......	67	91	ns§
4-chlorophenoxyacetic N,N-di-(2'-hydroxyethyl)-amide.............	58¶	51‡	ns§
4-chlorophenoxyacetyl chloride...............................	74	42	144†
2-(4'-chlorophenoxyacetamido)-2-(hydroxymethyl)-1,3-propanediol...	72	114	59†
gamma-(4-chlorophenoxy)-butyric acid*........................	71	113	112†
S-(4-chlorophenyl)-thioglycolic acid...........................	56	10	126†
crotyl 4-chlorophenoxyacetate*...............................	78¶	....	108†
2,4-dibromophenoxyacetic acid*..............................	76	94	53†
alpha, beta-dibromo-gamma-phenylpropionylchloride	57‖	10‡	ns§
3,5-dichloro-2-bromobenzoic acid.............................	57	144	49†
2,4-dichloro-5-bromophenoxyacetic acid*......................	74‖	61	35
2,4-dichlorophenoxyacetic piperidide..........................	71‖	8	25†
1-(2',4'-dichlorophenoxyacetamido)-naphthalene-4-sulphonic acid ...	76	43	ns§
2,4-dichlorophenoxyacetonitrile...............................	61	35	32†
N¹-(2,4-dichlorophenoxyacetyl)-betaine hydrazide, hydrochloride.....	61	0	ns§
N-2,4-dichlorophenoxyacetyl diethylamine......................	55‖	57	50†
N-2,4-dichlorophenoxyacetyl methylamine......................	64‖	71	ns§
gamma-(2,4-dichlorophenoxy)-butyric acid, ammonium salt*........	79	....	....
2,4-dichlorophenylglycine*..................................	60	6	20†
3-(2,5-dichlorophenyl)-thioglycolic acid chloride	61¶	10‡	0†
2,2-dimethyl-1,3-dioxolane-4-methanyl 4-chlorophenoxyacetate*.....	72	91	126
2-(2,4-dimethylphenoxy)-propionic acid*......................	65	0	−161†
3,5-dimethylpyrazole.......................................	50	0	0†
ethyl 3-hydroxy-2-naphthoate...............................	52‖	0‡	57†
ethyl 2-methyl-4,6-dichlorophenoxyacetate....................	72	35‡	25†
2-hydroxy-3-carboxy-5-bromotoluene.........................	55‖	0	18†
2-hydroxy-3-carboxy-5-iodotoluene...........................	66‖	0	24†
beta-hydroxyethyl 4-chlorophenoxyacetate*....................	74	159	86
N-(2-hydroxyethyl)-alpha-(2',4'-dichlorophenoxy)-acetamide*......	65	0	40†
N-(2-hydroxyethyl)-alpha-(2'-methyl-4'-chlorophenoxy)-acetamide*...	64	0	37†
beta-hydroxyethyl 2-methyl-4-chlorophenoxyacetate*.............	76	0	38
2-hydroxy-3-methylbenzoic acid..............................	74	27	14†
2-hydroxy-5-nitrobenzoic acid...............................	50	38	63†
2-methyl-4-bromo-6-carboxyphenoxyacetic acid.................	57	4	15†
3-methyl-4-chlorophenoxyacetamide...........................	62‖	23‡	25†

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.
‖ Tested as NH₄ salt after addition of sufficient NH₄OH to effect solution.
¶ Tested after addition of sufficient HCl to effect solution.

TABLE 2—*Continued*

Compound	Test		
	A	B	C
methyl 4-chlorophenoxyacetate*...............................	74	91	83†
2-methyl-5-chlorophenoxyacetic acid.........................	70	0	79†
3-methyl-4-chlorophenoxyacetic acid*........................	60	0	50†
2-methyl-4-chlorophenoxyacetic diethanolamide..............	63¶	2‡	63†
3-methyl-4-chlorophenoxyacetyl chloride.....................	62¶	30‡	47†
methyl 2,4-dibromophenoxyacetate*..........................	77	108	250†
methyl 2,4-dimethylphenoxyacetate*.........................	56	0	− 10†
2-methylphenoxyacetyl chloride..............................	67¶	0	18†
phenyl 4-chlorophenoxyacetate..............................	65‖	97‡	240†
phenyl 2,4-dichlorophenoxyacetate..........................	77	68‡	121†
2-(normal-propyl)-4-chlorophenoxyacetamide.................	72	0‡	− 175†
2,4,5-trichlorophenoxyacetanilide...........................	58	30	75†
2,4,5-trichlorophenoxyacetonitrile...........................	54¶	0‡	ns§
N-(2,4,5-trichlorophenoxyacetyl)-di-(tris-[hydroxymethyl]-methyl-aminomethyl)-carbinol, hydrochloride.....................	75	21	ns§

TABLE 3

ACTIVITIES OF COMPOUNDS: GROUP III

Compounds possessing 30–49% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A), listed in alphabetical order. Results of kidney-bean single-droplet water test (Test B) and kidney-bean single-droplet oil test (Test C) similarly expressed as percentage of activity of 2,4-dichlorophenoxyacetic acid in same tests.

COMPOUND	TEST		
	A	B	C
4-aminoazobenzene	41¶	o‡	74†
beta-(amylamino)-ethyl diphenylacetate, hydrochloride	36¶	o	−5†
2-amyl-4-chlorophenoxyacetic acid*	34‖	o‡	−152†
isoamyl 2,4-dimethylphenoxyacetate	34‖	4	83†
beta-bromoethyl 4-chlorophenoxyacetate*	47	116	107
2-bromophenylsulphamic acid	35‖	o	ns§
N-butylamine, mercuric chloride	30¶	o	ns§
normal-butyl 3-methylphenoxyacetate	30	17	53†
cacotheline	37	o	50†
N-4-carboxyphenyl N'-3-chlorophenyl urea	36‖	8‡	ns§
chloroacetamide	33	o	15†
4-chlorobenzoyl chloride	37‖	34	....
4-chlorophenoxyacetonitrile	45	17	84†
1-(4-chlorophenoxy)-2,4-epoxypropane*	41‖	21	61†
4-chlorophenylacetic acid*	38	22	−12†
N-(4-chlorophenyl)-glycine	34	o	−97
S-(4-chlorophenyl)-thioglycolic acid chloride	46	19	55†
S-(4-chlorophenyl)-thioglycolic butylamide	34	o‡	25†
2-cyanomethyl-4-chlorophenoxyacetic acid*	46	o	38†
N,N-(cyclopentamethylene)-dithiocarbamic acid, ammonium salt	31‖	o	37†
3,5-dibromo-2-aminobenzoic acid	33‖	o‡	35†
2,5-dichloro-aminobenzene, mercuri-chloride salt	33	o	−29†
2,4-dichloro-5-aminophenoxyacetic acid*	38	28	10†
2,4-dichlorocinnamic acid*	43‖	o‡	22†
2,4-dichloro-6-methylphenoxyacetamide	34	o‡	12
2,4-dichloro-5-nitrophenoxyacetic acid*	46	o	−10†
2,4-dichlorophenoxyacetic diethanolamide	49	41	93†
S-(2,5-dichlorophenyl)-thioglycolic acid	36	o‡	−10†
N,N-di-(alpha-hydroxy-beta,beta,beta-trichloroethyl)-urea	45‖	o	ns§
3,4-dimethylphenol	36	26	−4†
2,4-dimethylphenoxyacetic acid*	42	1	38†
3,4-dimethylphenoxyacetic acid*	38	o	83†
2,4-dimethylphenoxyacetyl chloride	30	35	48†
S-(2,4-dinitrophenyl)-thioglycolic acid	44	o	−81†
N,N-di-(tris-[hydroxy-methyl]-methyl)-ethylene diamine, dihydrochloride	48	o	34†
ethyl 2-chloromethyl-4-chlorophenoxyacetate*	36	o‡	39
2-ethyl-4-chlorophenoxyacetic acid*	43	39	45†
ethyl S-(4-chlorophenyl)-thioglycolate	48¶	10	−97†
2-hydroxy-3-carboxy-5-chlorotoluene	31‖	10	18†
4-hydroxy-3,5-dibromobenzoic acid	40	o	−15†
beta-hydroxyethyl 2,4-dichlorophenyl ether*	40	28	44
N4-(iodoacetyl)-sulphanilamide	37¶	o	ns§
beta-methyl beta-(butylamino)-propyl 4-hexyloxybenzoate, hydrochloride	46¶	....	75†
2-methyl-4-chloro-6-carboxyphenoxyacetic acid	40‖	o‡	15†

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.
‖ Tested as NH₄ salt after addition of sufficient NH₄OH to effect solution.
¶ Tested after addition of sufficient HCl to effect solution.

TABLE 3—*Continued*

Compound	Test		
	A	B	C
methyl 2-chlorophenoxyacetate*	32¶	0	61†
1-(2-methyl-4-chlorophenoxy)-2,3-epoxypropane*	33¶	0	0†
methyl 2,4-dichlorophenoxyacetate*	44	100‡	108
2-methylphenoxyacetic acid	32	0	ns§
4-nitrobenzoyl chloride	42‖	33	16†
mono-octyl acid orthophosphate	45‖	....	....
beta-(isopropylamino)-ethyl 2-butoxybenzoate hydrochloride	32	0	63†
normal propyl 2-methyl-4-chlorophenoxyacetate*	45‖	10‡	80
isopropyl N-phenyl carbamate*	46	16	90†
beta-pyridinesulphonic acid, barium salt	33	0	−19†
sulphamerazine	38	29	....
2,3,5-tribromobenzoic acid	41	31	24†
2,3,5-trichlorobenzoic acid	49‖	17	46†
N-(beta,beta,beta-trichloro-alpha-hydroxyethyl)-urea	32	34	62†
2,4,6-trichlorophenoxyacetic acid*	35‖	24	62†
2,4,5-trichlorophenoxyacetic 2'-nitroanilide	36	10	....
2,4,6-trichlorophenyl N-phenyl carbamate	49‖	64	3†
S-(2,4,5-trichlorophenyl)-thioglycolic amide	38	0‡	1
1-(3-trifluoromethylphenoxy)-2,3-epoxypropant*	43	12	38†
2,3,5-triiodobenzoic acid, ammonium salt*	34	55	....
N-(tris-[hydroxymethyl]-methyl), N-(beta-hydroxy, gamma-[tris-(hydroxymethyl)-methylamino]-propyl) 4-chlorophenoxyacetamide, hydrochloride	49	129	ns§

TABLE 4

ACTIVITIES OF COMPOUNDS: GROUP IV-A

Compounds showing less than 29% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A) and 50% or more of the activity of 2,4-dichlorophenoxyacetic acid by either the kidney-bean single-droplet water test (Test B) or the kidney-bean single-droplet oil test (Test C), listed alphabetically.

	Test		
COMPOUND	A	B	C
alpha-amino beta-(2,4-dichlorophenoxy)-propionamide.............	−38	56	−51†
alpha-amino beta-(3-nitro-4-hydroxyphenyl)-propionic acid, nitrate salt.................	−10	4	67†
aminotetrazole.................................	−8	20	52†
aniline.................................	−8	20	68†
benzyl carboxymethyl sulphone*......................./..........	−8	18	55†
3-bromo-6-nitrobenzoic acid*..........................	−3	0	50†
2-bromo-3-nitrobenzoic acid*..........................	0	82	160†
2-bromo-3-nitrobenzoic acid, ammonium salt*.....................	−4	99	....
beta-bromopropionic acid.........................	27	96	100†
beta-(butylamino)-ethyl 4-butoxybenzoate, hydrochloride..........	−1¶	0	94†
beta-(isobutylamino)-ethyl 4-butoxybenzoate, hydrochloride........	−27¶	0	63†
beta-(butylamino)-ethyl 4-ethoxybenzoate, hydrochloride..........	0	....	75†
beta-(butylamino)-ethyl 4-methoxybenzoate, hydrochloride..........	−20	....	88†
camphoroxime..................................	−8	1	110†
N4-(carbo-beta-chloro-ethoxy)-sulphanilamide.....................	−17	0	83†
2-carbomethyl-4-chlorophenoxyacetic acid*.....................	15	0	50†
2-carboxy-4-chlorophenoxyacetic acid.............................	−24	0	59†
2-carboxy-6-methylphenoxyacetic acid.............................	10	7	50†
2-carboxyphenoxyacetic acid.............................	−16	0	82†
2-carboxymethoxy-3,5-dichlorobenzoyl glycolic acid	6	21	55†
chloroacetic acid.................................	−22	41	79†
2-chloroaniline.................................	−25	12	59†
3-chloroaniline.................................	−32	0	82†
4-chloroaniline.................................	−30	0	75†
4-chlorobenzylmercaptan*.........................	9‖	12‡	55†
4-chlorobenzenesulphonyl chloride..............................	4‖	0	60†
4-chlorobenzyl-isothiourea, hydrochloride*.....................	10	19	72†
4-chloromandelic acid*.........................	29	32	53†
2-chloro-4-methylphenoxyacetic acid*.........................	11	0	70†
2-chloro-3-nitrobenzoic acid*.........................	−18	84	96†
2-chloro-5-nitrobenzoic acid*.........................	−3	0	100†
2-chlorophenoxyacetic acid*.........................	29	21	66
2-(2'-chlorophenyl)-phenoxyacetic acid*.........................	−6	0	96†
4-chlorothiophenol.........................	−6	0	75†
diazoaminobenzene.........................	14¶	0‡	78
2,4-dibromophenol.........................	1	40	120
dichloroacetic acid.........................	−38	0	56†
2,4-dichloroaniline.........................	−11	0	85†
2,5-dichloroaniline.........................	−23	27	61†
2,4-dichlorobenzyl carboxymethyl sulphone*.........................	29	4	61†
2,4-dichlorobenzoic acid*.........................	−1	0	80†
2,4-dichlorobenzyl isothiourea, hydrochloride*	23	0	50†
2,4-dichloro-6-carboxyphenoxyacetic acid.........................	1	14	83†
2,6-dichloro-4-nitrophenoxyacetic acid*.........................	3	13	50†
2,4-dichlorophenyl N-phenylcarbamate.........................	1	0	78†
2,5-dichlorophenylsulphamic acid.........................	28‖	0	50†

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.
‖ Tested as NH₄ salt after addition of sufficient NH₄OH to effect solution.
¶ Tested after addition of sufficient HCl to effect solution.

TABLE 4—*Continued*

Compound	Test		
	A	B	C
2,4-dihydroxypyrimidine	−28	68‡	ns§
2,4-dimethylphenol	14	9	64†
2,4-dinitrophenylacetic acid	−25	o	75†
N,N-di-(tris-[hydroxymethyl]-methyl) hexamethylenediamine, dihydrochloride	−23	o	75†
3-ethoxy-2-naphthoic acid	−27‖	o	52†
beta-(ethylamino)-butyl 4-ethoxybenzoate, hydrochloride	−19	9	56†
ethyl carbamate	−44	o	63†
ethyl beta-methyl-beta-(4-chlorophenyl)-glycidate*	26‖	o‡	84
3-ethyl-4-methyl-pyridine	−2	11	138
ethyl 2-propyl-4-chlorophenoxyacetate*	14	o‡	60†
2-fluorophenoxyacetic acid*	−51	8‡	72†
2-hydroxy-3-bromo-5-chlorobenzoic acid	23‖	20	75†
2-hydroxy-3-carboxy-5-nitrotoluene	−8	o	217†
N-(2-hydroxy-3-chloropropyl)-4-toluidine	17¶	o	74†
2-hydroxy-3,5-dinitrobenzoic acid	17	o	98†
4-iodobenzoic acid	o‖	o	100†
2-methoxyphenol	−4	o	76†
4-methoxyphenol	−22	54	....
beta-methyl, (beta-[amylamino]-propyl)-diphenylacetate, hydrochloride	−11¶	o	54†
2-methyl-5-chlorophenol	−15	o	52†
2-methyl-6-chlorophenol	−10	o	77†
2-methyl-4-chlorophenoxyfumaric acid*	7‖	o	108†
methyl N-3-chlorophenylcarbamate	18	o	57†
2-methyl-4,6-dichlorophenol	−2	o	100†
beta-methyl beta-(hexylamino)-propyl 4-ethoxybenzoate, hydrochloride	o¶	o	75†
methyl 2-methyl-6-chlorophenoxyacetate	14	33	62
4-methylphenoxyacetic acid	11	o	75†
methyl N-phenylthiocarbamate	11	61	
S-(2-methylphenyl)-thioglycolic acid	20	24	65†
4-methyl-4-trichloromethyl-2,5-cyclohexadien-1-one-O-carboxymethyl oxime*	−1	o	50†
2-nitrobutyl N-phenylcarbamate	−9	18	125†
1-phenyl-3-methyl-5-pyrazolone	−33	54	....
phthalic acid	25	o	55†
alpha-pinene	−13	o	89†
beta-(isopropylamino)-ethyl 4-butoxybenzoate, hydrochloride	−12	o	88
2-propyl-4-chlorophenoxyacetic acid*	11	23	67†
isopropyl 2,4-dimethylphenoxyacetate	24‖	14	72†
isopropyl 2-methyl-6-chlorophenoxyacetate	22	o	58
3-(normal propyl)-2-naphthoic acid	10‖	o‡	63†
isopropyl 2-propyl-4-chlorophenoxyacetate*	25‖	o‡	108
trichloroacetamide	−6	4	56†
trichloroacetic acid	−38	o	55†
trichloroacetyl chloride	−36	....	50†
2,4,5-trichlorobenzenesulphonamide	−7¶	o‡	61†
3,4,5-trihydroxybenzoic acid	−4	54	....
tris-(hydroxymethyl)-methyl beta,gamma-dibromo propylamine, hydrobromide	−5	o	88†
salicylic acid	26	54	....

TABLE 5

ACTIVITIES OF COMPOUNDS: GROUP IV-B

Compounds insufficiently soluble in water for corn-germination test (Test A) but exhibiting 50% or more of the activity of 2,4-dichlorophenoxyacetic acid by either kidney-bean single-droplet water test (Test B) or kidney-bean single-droplet oil test (Test C), listed alphabetically.

COMPOUND	TEST	
	B	C
allyl 4-chlorophenoxyacetate*	147‡	94
allyl 2,4-dichlorophenoxyacetate*	76‡	85
2-aminonaphthoic acid	0	66†
normal amyl 2,4-dichlorophenoxyacetate*	88‡	81
isoamyl 2,4-dichlorophenoxyacetate*	38‡	85
normal amyl N-alpha-naphthyl carbamate	13‡	75†
4,4'-(bis-chlorophenyl)-trichloromethyl methane	18‡	72†
1,1'-(bis-beta-naphthol)-phenyl methane	20‡	67†
2-bromo-3,5-dichlorobenzamide	0‡	89†
2-bromo-3,5-dichlorobenzanilide	7	89†
2-bromo-3,5-dichlorobenzo-2'-bromoanilide	0‡	72†
2-bromo-3,5-dichlorobenzo-3'-bromoanilide	0‡	52†
2-bromo-3,5-dichlorobenzo-4'-bromoanilide	13‡	78†
2-bromo-3,5-dichlorobenzo-3'-chloroanilide	1	67†
2-bromo-3,5-dichlorobenzo-2',4'-dichloroanilide	19‡	100†
2-bromo-3,5-dichlorobenzo-3'-toluidide	20‡	61†
2-bromo-3,5-dichlorobenzoyl chloride	63‡	110†
beta-bromoethyl 2,4-dibromophenoxyacetate*	59	100
beta-bromoethyl 2,4-dichlorophenoxyacetate*	126‡	117
4-bromophenoxyacetamide	139	ns§
N-3-bromophenyl N'2-chlorophenyl urea	0‡	59†
N-3-bromophenyl N'3-chlorophenyl urea	0‡	60†
butyl 2,4-dichlorophenoxyacetate*	50	111
isobutyl 2,4-dichlorophenoxyacetate*	133‡	64
N-carbethoxy N'-3-chlorophenyl urea	1‡	77†
beta-chloroethyl 4-chlorophenoxyacetate*	117	97†
beta-chloroethyl 2,4-dibromophenoxyacetate*	57	77†
beta-chloroethyl 2,4-dichlorophenoxyacetate*	49	112†
beta-chloroethyl 2-methyl-4-chlorophenoxyacetate*	15	99†
beta-chloroethyl N-(alpha-naphthyl)-carbamate	8‡	67†
beta-chloroethyl N-phenyl carbamate	19	75†
4-chlorophenoxyaceto-4-anisidide	0‡	81†
4-chlorophenoxyaceto-2-bromoanilide	21‡	104†
4-chlorophenoxyaceto-3-bromoanilide	88‡	18†
4-chlorophenoxyaceto-4-bromoanilide	35‡	94†
4-chlorophenoxyaceto-2-chloroanilide	54‡	·········
4-chlorophenoxyaceto-3-chloroanilide	99‡	99†
4-chlorophenoxyaceto-2',4'-dimethylanilide	0‡	60†
4-chlorophenoxyaceto-4'-ethoxyanilide	0‡	109†
N'-(4-chlorophenoxyacetyl) N²-phenyl hydrazine	55‡	86†
4-chlorophenoxyaceto-4'-iodoanilide	36‡	75†
4-chlorophenoxyaceto-3'-nitroanilide	0‡	108†
4-chlorophenoxyaceto-4'-toluidide	64‡	155†
4-chlorophenoxyaceto-4'-xenylamide	0‡	89†
gamma-(4-chlorophenoxy)-butyronitrile*	10	62†
4-chlorophenyl 4'chlorophenoxyacetate	136‡	85†
N-4-chlorophenyl N'-2-chlorophenyl urea	43‡	71†
4-chlorophenyl 2',4'-dichlorophenoxyacetate	83	92†

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.

TABLE 5—*Continued*

Compound	Test B	Test C
N-3-chlorophenyl N′,N′-(cyclopentamethylene) urea	55‡	64†
N-3-chlorophenyl N′-phenyl urea	0‡	64†
S-(4-chlorophenyl)-thioglycollic 2′-bromoanilide	0‡	60†
S-(4-chlorophenyl)-thioglycollic 3′-bromoanilide	29‡	70†
4-chlorophenyl 2′,4′,5′-trichlorophenoxyacetate	69‡	185†
2,6-dibromobenzoquinone-4-chlorimide	0	63†
2,4-dichlorobenzylsulphonyl chloride*	28‡	107†
1,3-di-(4-chlorophenoxyacetamido)-benzene	0	75†
1,4-di-(4-chlorophenoxyacetamido)-benzene	0	62†
4,4′-di-(4-chlorophenoxyacetamido)-biphenyl	0	50†
2,4-di-(4-chlorophenoxyacetamido)-toluene	0	88†
2,4-dichlorophenoxyacetanilide	88‡	164†
2,4-dichlorophenoxyacetic beta-aminoethylamide	132‡	ns§
2,4-dichlorophenoxyaceto-4′-anisidide	0‡	136†
2,4-dichlorophenoxyaceto-2′,5′-dichloroanilide	0‡	90†
2,4-dichlorophenoxyaceto-2′,4′-dimethylanilide	37‡	113†
N′-(2,4-dichlorophenoxyacetyl) N²-(2′,4′-dinitrophenyl) hydrazine	57‡	ns§
2,4-dichlorophenoxyacetic hydrazide	134‡	ns§
2,4-dichlorophenoxyaceto-beta-naphthalide	28‡	71†
2,4-dichlorophenoxyaceto-4′-toluidide	82‡	41†
2,4-dichlorophenoxyaceto-2′-xenylamide	54‡	75†
4-(2,4-dichlorophenoxyacetamido)-azobenzene	18‡	81†
2,4-dichlorophenoxyacetyl aminoguanidine	73‡	17†
2,4-dichlorophenoxyacetyl bromide	57‡	−17†
2,4-dichlorophenoxyacetyl (dimethyl, methylol)-methylamide	7‡	67†
S-(2,4-dichlorophenoxyacetyl) isothiourea	72‡	
2,4-dichlorophenoxyacetyl methyl-isothiourea	56‡	ns§
gamma-(2,4-dichlorophenoxy)-butyric acid*	96	29†
gamma-(2,4-dichlorophenoxy)-butyronitrile*	3	64†
2,4-dichlorophenyl 4′-chlorophenoxyacetate	132‡	87†
2,4-dichlorophenyl 2′,4′-dichlorophenoxyacetate	68‡	112†
N-(2,5-dichlorophenyl) N′-phenyl urea	18‡	66†
S-(2,5-dichlorophenyl)-thioglycolic amide	0‡	60†
4,4′-di-(2,4-dichlorophenoxyacetamide)-biphenyl	0	52†
1,4-di-(2,4-dimethylphenoxyacetamide)-benzene	0‡	90†
2,4-di-(2,4-dimethylphenoxyacetamide)-toluene	0‡	140
2,4-dichlorophenyl-2′,4′,5′-trichlorophenoxyacetate	113	ns§
2,4-dichloropropyl 4′-chlorophenoxyacetate*	126‡	229
beta,gamma-dichloropropyl 2′,4′-dibromophenoxyacetate*.	79‡	74
2,3-dichloropropyl 2′,4′-dichlorophenoxyacetate*	130‡	70
beta-diethylaminoethyl 2,3,5-triiodobenzoate*	18	50†
3,3′-dimethyl-4,4′-(4-chlorophenoxyacetamide)-biphenyl	0	81†
3,3′-dimethyl-4,4′-(2-methylphenoxyacetamido)-biphenyl	0	62†
1,3-di-(2-methylphenoxyacetamide)-benzene	0	69†
1,4-di-(2-methylphenoxyacetamide)-benzene	0	88†
4,4′-di-(2-methylphenoxyacetamide)-biphenyl	0	81†
4,4′-di-(2,4-dimethylphenoxy-acetamide)-diphenyl	0‡	90†
N-4-ethoxyphenyl N′-phenyl urea	52‡	86†
ethyl 2-bromo-3,5-dichlorobenzoate	0‡	85†
ethyl 4-bromophenoxyacetate*	90	80†
ethyl 4-chlorophenoxyacetate*	88	150†
2-ethylhexyl 2′,4′-dichlorophenoxyacetate*	55	72
methallyl 4-chlorophenoxyacetate*		107†
2-methoxy-4-methylphenyl N-(alpha-naphthyl)-carbamate	0‡	105†
methyl 2-bromo-3-nitrobenzoate*	90	75†
4-(2-methyl-4-chlorophenoxyacetamido)-azobenzene	0‡	67†
2-methyl-6-chlorophenoxyaceto-2′,5′-dichloroanilide	0	57†
2-methyl-4-chlorophenyl 2′,4′-dichlorophenoxyacetate	25‡	100†

TABLE 5—*Continued*

COMPOUND	TEST B	TEST C
1-methyl-2,4-di-(2,4-dichlorophenoxyacetamide)-benzene.....	o	65†
methyl N-4-nitrophenylcarbamate.......................	9‡	53†
methyl 2,4,5-trichlorophenoxyacetate...................	83	200†
1-(betanaphthyl) N-piperidylphenyl-methane..............	31‡	59†
2-nitrobutyl 2,4,5-trichlorophenoxyacetate	88	390†
4-nitro-dimethylaniline.................................	11‡	105†
normal-octyl 2,4-dichlorophenoxyacetate*...............	92‡	88
pentachlorophenyl 2,4,5-trichlorophenoxyacetate...........	3‡	210†
N-phenyl-N',N'-cyclopentamethylene urea	63	36†
phenyl N-phenylcarbamate............................	o	59†
phenyl 2,4,5-trichlorophenoxyacetate...................	61‡	173†
isopropyl 2,4-dichlorophenoxyacetate*..................	94‡	68
3-isopropoxy-2-naphthoic acid..........................	o	59†
N,N'-di-(3-tolyl) urea	o‡	85†
2,4,5-tribromo-3,5-dimethylphenoxyacetic acid.............	o‡	74†
2,4,6-tribromophenylacetate..........................	o	71†
2,4,5-trichlorobenzamide..............................	64‡	ns§
trichloroethyl 2,4-dibromophenoxyacetate*..............	59‡	90
beta-trichloroethyl 2,4-dichlorophenoxyacetate*..........	140‡	62
2,4,5-trichlorophenoxyacetic acid*.....................	93	236†
2-(2',4',5'-trichlorophenoxyacetamido)-anthraquinone........	51‡	175†
2,4,5-trichlorophenoxyaceto-4'-bromoanilide...............	51‡	83†
2,4,5-trichlorophenoxyaceto-4'-methoxyanilide............	37‡	128†
2,4,5-trichlorophenoxyaceto-beta-naphthalide.............	o‡	218†
2,4,6-trichlorophenoxyaceto-4'-sulphonaphthalide..........	45‡	57†
2,4,5-trichlorophenoxyaceto-3'-toluidide..................	11‡	130†
2,4,5-trichlorophenoxyacetyl chloride....................	117‡	207†
N'-(2,4,5-trichlorophenoxyacetyl)-para-nitrophenylhydrazine..	75‡	ns§
2,4,6-trichlorophenyl 4'-chlorophenoxyacetate.............	66‡	69†
2,4,6-trichlorophenyl 2',4'-dichlorophenoxyacetate	85‡	46†
2,4,6-trichlorophenyl 2',4',5'-trichlorophenoxyacetate........	77	190†
N-(3-[trifluoromethyl]-phenyl)-alpha-(4-chlorophenoxy)-acet-amide.	3‡	51†
N-(3-[trifluoromethyl]-phenyl)-alpha-(2,4,5-trichlorophloro-phenoxy)-acetamide*.	11‡	70†
2,3,5-triiodobenzoic acid*............................	51	49†
2,3,5-triiodobenzoyl chloride...........................	o	106†
1-(tris-[hydroxymethyl]-methylamino) 2,4-dinitrobenzene....	o	59†
N-(para-xenyl)-2,4-dichlorophenoxyacetamide.............	61‡	ns§

TABLE 6

ACTIVITIES OF COMPOUNDS: GROUP IV-C

Other compounds tested in comparison with 2,4-dichlorophenoxyacetic acid by the corn-germination test (Test A), single-droplet water test (Test B), and single-droplet oil test (Test C), listed alphabetically.

COMPOUND	TEST		
	A	B	C
2-acetoxyl-ethyl N-(alpha-naphthyl)-carbamate	....	39‡	....
2-acetoxyl-ethyl N-phenylcarbamate	....	15‡	....
2-acetyl-4-chlorophenoxyacetic acid*	6	o	−20†
2-allyl-4-chlorophenoxyacetic acid*	11	o	−21†
allyl N-(alpha-naphthyl)-carbamate	ns§	o‡	2
allyl N-phenylcarbamate	8	8	36
allyl 4-tolyl sulphone*	8	o	−47
1-aminoanthraquinone	ns§	o	ns§
2-aminoanthraquinone	ns§	o	ns§
4-aminobenzyl tris-(hydroxymethyl)-methylamine, dihydrochloride	8	o	ns§
2-amino-3,5-dichlorobenzoic acid	−17	18	48
2-amino-ethylsulphuric acid	14	27	ns§
1-amino-8-naphthol-3,6-disulphonic acid	2	o	o†
1-amino-2-naphthol-4-sulphonic acid	− 1‖	o	ns§
4-aminophenol	13	47	....
2-aminophenoxyacetic acid	−41	7	ns§
4-aminophenylacetic acid	23	o‡	ns§
2-aminopyridine	19	o	−50†
2-aminothiazole	−20	o	21†
beta-(amyl-amino)-ethyl 4-butoxy-benzoate, hydrochloride	5	....	25†
isoamylformate	17	47	42
normal-amyl 2-methylphenoxyacetate	22¶	o‡	35†
isoamyl N-(alpha-naphthyl)-carbamate	ns§	o	ns§
4-tertiary-amylphenol	5	9	....
normal-amyl N-phenylcarbamate	ns§	11‡	−46
isoamyl N-phenylcarbamate	ns§	o‡	− 1
4-arsenophenoxyacetic acid	22	9	28§
benzoic acid	20	33	21†
4-(benzylamino)-phenol, hydrochloride	−23¶	o	ns§
benzyl normal-butyl sulphone*	− 6	7	ns§
benzyl carboalloxymethyl sulphone*	11	13	14
benzyl carbomethoxymethyl sulphone*	− 3	o	− 5†
N-benzyl-N,N'-(di-tris-[hydroxy-methyl]-methyl) 2-hydroxy-1,3-di-aminopropane	− 7¶	....	o†
benzyl ethyl sulphone*	− 4	o	ns§
benzyl methyl sulphone*	− 1	o	ns§
benzyl 4-tolyl sulphone*	ns§	o‡	ns§
benzyl tris-(hydroxy-methyl)-methyl amine	− 1¶	o	....
1,3-bis-(tris-[hydroxy-methyl])-methylamino-2-prop_nol, dihydrochloride	− 4	36	ns§
2-bromobenzamide	15	25	−21†
2-bromo-benzanilide	ns§	o	5
2-bromo-benz-2,4-dichloroanilide	ns§	12‡	− 6
2-bromobenzoic acid	17	o	ns§
3-bromobenzoic acid	25	o	43†
4-bromobenzoic acid, ammonium salt	− 7	o	−145†
4-bromobenzonitrile	1	7	5†
2-bromo-4-tertiary-butyl-phenoxyacetic acid*	10	o	24†
2-bromo-3,5-dichloro-benzoic butylamide	ns§	14‡	44

* Compounds prepared at Ohio State University.
† Tributylphosphate used as co-solvent.
‡ Not entirely soluble.
§ Not sufficiently soluble for testing.
‖ Tested as NH₄ salt after addition of sufficient NH₄OH to effect solution.
¶ Tested after addition of sufficient HCl to effect solution.

TABLE 6—*Continued*

COMPOUND	Test A	B	C
2-bromo-3,5-dichloro-benzoic 4'-chloro-anilide	ns§	0‡	44
beta-bromoethyl amine	− 7	0	....
beta-(bromoethyl) 4-ethoxy-thiolbenzoate	ns§	....	25
beta-bromoethyl 2-methyl-4-chlorophenoxyacetate*	ns§	0‡	56
2-bromo-4-nitrobenzoic acid*	9	0	−10†
2-bromo-5-nitrobenzoic acid*	....	17	....
2-bromo-5-nitrobenzoic acid, ammonium salt*		0	
3-bromo-4-nitrobenzoic acid*	18	0	0†
3-bromo-5-nitrobenzoic acid*	− 4	0	35†
4-bromophenol	6	20	−114†
2-bromophenoxyacetic acid*	0	0	38†
4'-bromophenoxyacetic 4-bromoanilide	ns§	10	ns§
4'-bromophenoxyacetic 4-chloroanilide	ns§	30‡	ns§
4'-bromophenoxyacetic 2,5-dichloroanilide	ns§	32‡	ns§
3-bromophenylammonium fluoborate	− 1	21	....
4-bromophenylammonium fluoborate	6	6	....
N-2-bromophenyl N'-2-chlorophenyl urea	ns§	42‡	ns§
N-4-bromophenyl N'-3-chlorophenyl urea	ns§	45‡	40
N-2-bromophenyl N'-3-chlorophenyl urea	ns§	0‡	7
N-4-bromophenyl N'-2-chlorophenyl urea	ns§	31‡	−42
N-4-bromophenyl-dithiocarbamic acid, ammonium salt	− 2	0‡	....
4-bromophenyl N-(alpha-naphthyl)-carbamate	ns§	0	ns§
2-bromo-4-phenylphenoxyacetic acid*	− 1	29	−18†
4-bromophenyl N-phenylcarbamate	ns§	0	31
N-2-bromophenyl N'-phenyl urea	ns§	0‡	32
N-3-bromophenyl N'-phenyl urea	ns§	0‡	−11
N-4-bromophenyl N'-phenyl urea	ns§	0‡	2
3-bromophenyl sulphamic acid	12	0	ns§
N-(3-bromophenyl)-alpha, alpha, alpha, trichloro-acetamide	ns§	7‡	−23
beta-(butyl-amino)-ethyl 2-(butoxy)-benzoate, hydrochloride	− 4	0	12†
beta-(butyl-amino)-ethyl diphenyl-acetate, hydrochloride	−20¶	0	ns§
beta-(butyl-amino)-ethyl 4-heptyloxy benzoate, hydrochloride	20¶	....	25†
beta-(butyl-amino)-ethyl 4-propoxybenzoate, hydrochloride	−18¶	....	0†
beta-(butyl-amino)-ethyl 2-(thiobutoxy)-benzoate	7	16	19†
2-secondary-butyl-4-chlorophenoxyacetic acid*	26‖	0	34†
N-butyl-dithiocarbamic acid, mercury salt	ns§	15‡	ns§
normal-butyl N-(alpha-naphthyl)-carbamate	ns§	0‡	21
isobutyl N-(alpha-naphthyl)-carbamate	ns§	5‡	8
4-tertiary-butylphenol	−13	0	....
normal-butyl N-phenylcarbamate	ns§	18	44
isobutyl N-phenylcarbamate	ns§	15	45
tertiary-butyl N-phenylcarbamate	ns§	43‡	ns§
N-(normal-butyl) N'-phenyl thiourea	− 4	0	46†
N-(normal-butyl)-2,4,5-trichlorophenoxy-acetamide	ns§	0	....
4-carbethoxy-6-methoxyquinoline	−25	8	47†
N-carbethoxy N'-phenyl urea	ns§	0	ns§
alpha-(carbo-normal-butoxy)-ethyl N-(alpha-naphthyl)-carbamate	ns§	24	− 8
alpha-(carbo-isopropoxy)-ethyl N-(alpha-naphthyl)-carbamate	ns§	19	22
O-(2-carboxymethoxy-benzoyl)-glycolic acid	17	20	38†
O-(2-carboxymethoxy-3-methyl-5-chloro-benzoyl)-glycolic acid	27	0‡	23†
N-(carboxymethyl)-dithiocarbamic acid, ammonium salt	5	12‡	ns§
N-(4-carboxy-methyl-phenyl)-dithiocarbamic acid, sodium salt	0	0	ns§
2-carboxy-6-methylphenyl N-phenyl-carbamate	20‡	12‡	ns§
N-(4-carboxy-phenyl)-dithiocarbamic acid, ammonium salt	6	0	ns§
4-carboxyphenylglycine	17	0	−17†
ortho-carboxyphenyl N-(alpha-naphthyl)-carbamate	ns§	2	ns§
N-(4-carboxyphenyl) N'-(alpha-naphthyl) urea	−22	34	ns§
4-carboxyphenyl N-phenylcarbamate	ns§	0‡	ns§
S-(4-carboxyphenyl)-thioglycolic acid	−18‖	0	−19†

TABLE 6—*Continued*

COMPOUND	Test		
	A	B	C
N-t-(beta-carboxy-propionyl)-sulphanilamide.	−19‖	o	ns§
catechol.	−34	o	12†
chloroacetyl chloride.	− 6	3	49†
4-chloroanisole*.	−31	o	− 8†
2-chlorobenzaldehyde O-carboxymethyl oxime*.	4	8	10†
2-chlorobenzaldoxime*.	20	1	5†
4-chlorobenzamide.	4	7	ns§
4-chlorobenzenesulphonamide.	ns§	o	44
4-chlorobenzoic acid.	6	o	ns§
bis-(4-chlorobenzyl) disulphide*.	ns§	27‡	55
S-(4-chlorobenzyl)-thioglycolic acid*.	− 9	25	−15†
bis-(4-chlorobenzyl) sulphide*.	ns§	18‡	36
4-chlorobenzylsulphonylacetic acid*.	2	5	−15†
4-chlorocinnamic acid*.	26‖	23‡	43†
highly chlorinated 1,5-dihydroxynaphthalene.	ns§	o	13
beta-chloroethyl 2-propyl-4-chlorophenoxyacetate*.	ns§	o‡	46
chlorohydroquinone.	18	47	....
chlorohydroquinone-O, O-diacetic acid*.	1	o	....
4-(chloromercuri)-phenol.	28‖	6	ns§
4-(chloromercuri)-phenoxyacetic acid.	ns§	18‡	ns§
2-chloromethyl 4'-chlorophenoxyacetic acid*.	17	o	38†
2-chloro-4-methyl-6-methoxyquinoline.	ns§	o‡	ns§
2-chloro-4-methylquinoline.	ns§	o	20
7-chloro-1-naphthoxyacetic acid*.	13	16	−12†
alpha-chloronaphthylacetic acid mixt.*.	ns§	12	14
4-chlorophenetole*.	−8	o	−29†
1-(4'-chlorophenoxyacetamido)-naphthalene.	ns§	12‡	ns§
2-(4'-chlorophenoxyacetamido)-naphthalene.	ns§	4‡	ns§
4'-chlorophenoxyacetic 2,5-dichloroanilide.	ns§	47‡	ns§
4-chlorophenoxyacetic diethylamide.	....	44	....
4-chlorophenoxyacetic piperidide.	17	7	ns§
4'-chlorophenoxyacetic 2-nitroanilide.	ns§	14‡	ns§
4'-chlorophenoxyacetic 2,4,6-trichloroanilide.	ns§	o‡	19
4-chlorophenoxy 4'-chlorophenyl acetic acid*.	−19	25	o†
4-chlorophenoxyfumaric acid*.	o	o	ns§
alpha-(4-chlorophenoxy)-heptylic acid*.	22	o	−191†
beta-(4-chlorophenoxy)-propionic acid*.	2	30	12†
beta-(4-chlorophenoxy)-propionitrile*.	−38	24	24†
4-chlorophenylammonium fluoborate.	10	61	....
N-2-chlorophenyl N'-normal-butyl urea.	....	10‡	3
N-3-chlorophenyl N'-normal-butyl urea.	23	5‡	20†
N-2-chlorophenyl N-4-carboxyphenyl urea.	ns§	21	....
N-(3-chloro-phenyl)-alpha-chloro-acetamide.	−21	o	32†
N-(4-chloro-phenyl)-alpha-chloro-acetamide.	5¶	o‡	−51†
N-3-chlorophenyl N'-2-chlorophenyl urea.	ns§	24‡	−27
N-4-chlorophenyl N'-3-chlorophenyl urea.	ns§	o‡	ns§
N-2-chlorophenyl N',N'-cyclopentamethylene urea.	ns§	o‡	23
N-4-chlorophenyl dithiocarbamic acid, ammonium salt.	19	o	....
2-chloro-1,4-phenylene-bis-(N-phenyl carbamate).	ns§	o	ns§
N-(2-chlorophenyl)-glycine.	−16	7	38†
N-2-chlorophenyl N'-beta-hydroxyethyl urea.	ns§	o‡	−21
N-3-chlorophenyl N'-beta-hydroxyethyl urea.	ns§	23‡	−27
3-chlorophenyl isocyanate.	ns§	o‡	o
N-(2-chlorophenyl) N'-(alpha-naphthyl) urea.	ns§	o‡	ns§
N-(4-chlorophenyl) N'-(alpha-naphthyl) urea.	ns§	19‡	ns§
2-(4'-chlorophenyl)-phenoxyacetic acid*.	ns§	o	ns§
N-(2-chlorophenyl) N'-phenyl urea.	5	4	40
N-(2-chlorophenyl) N'-phenyl urea.	ns§	o‡	28
N-(2-chlorophenyl) N'-phenyl thiourea.	ns§	o‡	ns§

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N-(3-chlorophenyl) N′-phenyl thiourea	ns§	10‡	14
N-(4-chlorophenyl) N′-phenyl thiourea	ns§	0‡	22
3-chlorophenyl sulphamic acid, sodium salt	13	0	6†
4-chlorophenyl sulphamic acid	−11	0	ns§
S-(2-chlorophenyl)-thioglycolic acid	5	0	7†
S-(4-chlorophenyl)-thioglycolic amide	28	6‡	40†
S-(4-chlorophenyl)-thioglycolic anilide	ns§	32‡	40
S-(4-chlorophenyl)-thioglycolic 4′-bromoanilide	ns§	0‡	35
S-(4-chlorophenyl)-thioglycolic 4′-phenetidide	ns§	0‡	38
S-(4-chlorophenyl)-thioglycolic 3′-toluidide	ns§	23‡	45
N-2-chlorophenyl urea	ns§	0†	ns§
N-3-chlorophenyl urea	ns§	0†	− 6
N,N′-di-(2-chlorophenyl) urea	ns§	28†	ns§
N,N′-di-(3-chlorophenyl) urea	ns§	0†	− 6
cinnamic amide	12	6	ns§
cinnamoyl chloride	9	27	−115†
2-cresol	− 6	0	−134
3-cresol	0	2	−20
4-cresol	−29	5	−32
4-cresoxyacetyl chloride	− 3	31	25†
cyanoacetamide	17	8	28†
2-cyclohexyl-4-chlorophenoxyacetic acid*	18‖	8†	33†
decyl carboxymethyl sulphide*	13‖	0†	−10†
normal-decyl carboxy-methyl sulphone*	10	0	−53†
di-(2-acetoxyethyl) sulphone*	−13	23	29
2,6-diaminopyridine, monohydrochloride	−24	28	ns§
2,6-dibromo-4-carboxyphenyl N-phenylcarbamate	ns§	23	35
alpha, beta-dibromo-dihydrocinnamic acid	3	6	− 5†
4,6-dibromo-1,3-dihydroxy-benzene	18	0	15†
2,6-dibromo-4-methyl-phenoxyacetic acid	7	0	14
2,4-dibromophenyl N-phenylcarbamate	ns§	0	3
alpha, beta-dibromo-gamma-phenylpropionamide	ns§	18‡	−14
di-(2-butyroxyethyl) sulphone*	− 5	0	3
2,5-dichloro-4-aminobenzenesulphonic acid	8	0	27†
2,4-dichloroanisole*	−79	0	15†
2,6-dichlorobenzeneoneindophenol, sodium salt	−28	0	0†
2,5-dichlorobenzenesulphonamide	−39¶	11	−33†
2,5-dichlorobenzenesulphonyl chloride	ns§	0‡	48
2,4-dichlorobenzyl carboxymethyl sulphide*	15	32	28†
bis-(2,4-dichlorobenzyl) disulphide*	ns§	2‡	2
2,4-dichlorobenzyl mercaptan*	ns§	0‡	23
di-(2,4-dichlorobenzyl) sulphide*	ns§	12‡	27
di-(2,4-dichlorobenzyl) sulphone*	ns§	23‡	23
5,7-dichlorocoumaran-3-one	....	....	15
2,4-dichloro-N-chloroacetanilide*	6	12	0†
2,6-dichloro-3-ethyl-4-methyl-pyridine	ns§	0	44
2,4-dichloromandelic acid*	13‖	5	43†
2,6-dichloro-4-methyl-5-ethyl-nicotinamide	−19	0	ns§
2,6-dichloro-4-methylphenoxyacetic acid*	− 1	0	50
2,4-dichloro-6-methyl-phenoxyacetyl chloride	ns§	0	44
2,4-dichloro-1-naphthoxyacetic acid*	....	0	−40
2,4-dichlorophenetole*	0	0	−37†
2,4-dichlorophenol	13	0	−16†
1-(2′,4′-dichlorophenoxyacetamido)-anthraquinone	ns§	45‡	ns§
2-(2′,4′-dichlorophenoxyacetamido)-anthraquinone	ns§	45‡	ns§
2,6-dichlorophenoxyacetic acid*	15	15	15†
3,5-dichlorophenoxyacetic acid*	17	0	24†
2,4-dichlorophenoxyaceto-4′-bromoanilide	ns§	6‡	20
2,4-dichlorophenoxyaceto-4′-chloroanilide	ns§	23‡	3

TABLE 6—*Continued*

Compound	Test A	B	C
2,4-dichlorophenoxyaceto-4'-phenetidide	11s§	27‡	ns§
2,4-dichlorophenoxyacetic N-(beta-hydroxyethyl)-amide	ns§	37‡	ns§
2,4-dichlorophenoxyaceto-alpha-naphthalide	ns§	31‡	ns§
2,4-dichlorophenoxyaceto-2'-nitroanilide	ns§	38‡	ns§
2,4-dichlorophenoxyaceto-3'-nitroanilide	ns§	4‡	ns§
N¹-(2',4'-dichlorophenoxyacetyl) N²-(para-nitrophenyl) hydrazine	ns§	19‡	ns§
2,4-dichlorophenoxyacetic (2'-pyridyl)-amide			28
2,4-dichlorophenoxyaceto-2',4',6'-trichloroanilide	ns§	27‡	ns§
2-(2',4'-dichlorophenoxyacetamido)-naphthalene-6,8-disulphonic acid		36	128
N¹-(2',4'-dichlorophenoxyacetyl) N¹-phenylsemicarbazide	ns§	21‡	ns§
2,4-dichlorophenoxy para-chlorophenylacetic acid*	4	41	6†
1-(2',4'-dichlorophenoxy)-2,3-epoxypropane*	ns§	0	0
2,4-dichlorophenoxyfumaric acid*	−15	0	ns§
alpha-(2,4-dichlorophenoxy)-heptylic acid*	17	0‡	−107†
beta-(2,4-dichlorophenoxy)-propionic acid	−22	0	6†
alpha-(2,4-dichlorophenoxy)-stearic acid*	ns§	0	10
N-(2,4-dichlorophenyl)-alpha-chloroacetamide	9‖	0‡	22†
N-(2,5-dichlorophenyl)-alpha-chloroacetamide	ns§	0‡	22
2,4-dichlorophenyl alpha-chloroacetate	−22	22	−15†
N-2,5-dichlorophenyl chlorophenyl urea	ns§	0‡	ns§
N-2,5-dichlorophenyl N'-3-chlorophenyl urea	ns§	0‡	ns§
N-(2,5-dichlorophenyl)-glycine*	−10	19	40†
2,4-dichlorophenyl 2'-methyl-4'-chlorophenoxyacetate	ns§	10	0
2,4-dichlorophenyl N-(alpha-naphthyl)carbamate	ns§	0‡	ns§
N-(2,4-dichlorophenyl) N'-(alpha-naphthyl) urea	ns§	27	ns§
N-(2,4-dichlorophenyl) N'-phenyl thiourea	ns§	0‡	17
N-(2,5-dichlorophenyl) N'-phenyl thiourea	ns§	0‡	−38
N-(2,4-dichlorophenyl) N'-phenyl urea	ns§	24‡	31
S-(2,4-dichlorophenyl)-thioglycolic acid	11	0	10†
S-(2,5-dichlorophenyl)-thioglycolic anilide	ns§	0‡	20
S-(2,5-dichlorophenyl)-thioglycolic 4'-anisidide	ns§	28‡	10
S-(2,5-dichlorophenyl)-thioglycolic 2'-bromoanilide	ns§	0‡	10
S-(2,5-dichlorophenyl)-thioglycolic 3'-bromoanilide	ns§	6‡	20
S-(2,5-dichlorophenyl)-thioglycolic 3'-chloroanilide	ns§	10‡	−10
S-(2,5-dichlorophenyl)-thioglycolic 2',4'-dichloroanilide	ns§	10	28
S-(2,5-dichlorophenyl)-thioglycolic diphenylamide	ns§	9‡	20
S-(2,5-dichlorophenyl)-thioglycolic 4'-toluidide	ns§	38‡	14
2-(beta, gamma-dichloropropyl)-4-chlorophenoxyacetic acid*	−28	0	−43†
3,5-dichlorosalicylic acid		0	−106†
2,4-dichlorothiophenol	−10‖	29	26†
1,3-di-(2',4'-dichlorophenoxyacetamido)-benzene	ns§	29	26†
1,4-di-(2',4'-dichlorophenoxyacetamido)-benzene	ns§	0	− 5
1,3-di-(2',4'-dimethylphenoxyacetamido)-benzene	ns§	0	12
N-(alpha-[diethylamino]-diethylacetyl) urea	ns§	0‡	47
N,N-diethyldithiocarbamic acid, sodium salt	8	0	4†
2,6-dihydroxy-3-ethyl-4-methyl-5-carbethoxypyridine	− 7	8	ns§
2,6-dihydroxy-3-ethyl-4-methyl-5-cyanopyridine	−10	11	− 5†
2,6-dihydroxy-3-ethyl-4-methylpyridine	−24	21	0†
2,6-dihydroxy-4-methyl-5-cyanopyridine, ammonium salt	−22	0	ns§
1,5-dihydroxynaphthalene	2	9	ns§
2,5-dihydroxytoluene	ns§	39	25
3,4-diiodobenzoic acid*	−26	−15	0†
2,4-diiodophenoxyacetic acid*	− 4	11	
2,4-diketo-1,2,3,4-tetrahydro-pyrimidine-6-acetic acid	23	0	11†
2,4-dimethyl-3,5-dicarbethoxy-pyrrole	13	0	ns§
3,5'-dimethyl-4,4'-di-(2'',4''-dichlorophenoxyacetamido)-biphenyl	ns§	0‡	10
3,3'-dimethyl-4,4'-di-(2'',4''-dimethylphenoxyacetamido)-biphenyl	ns§	0‡	14
3,5-dimethylisoxazole	0	0	19†
2,5-dimethylphenol	5	0	−159

TABLE 6—*Continued*

Compound	Test		
	A	B	C
2,4-dimethylphenoxyacetamide	−15	o	− 4†
2,4-di-(2'-methylphenoxyacetamido)-toluene	ns§	o‡	38
1-(2',4'-dimethylphenoxyacetamido)-2,4-dichlorobenzene	ns§	o‡	5
1-(2',4'-dimethylphenoxyacetamido)-2,4,6-trichlorobenzene	ns§	2‡	48
2,5-dimethylphenoxyacetic acid*	− 1	8	30†
3,5-dimethylphenoxyacetic acid*	3	13	5†
2,4-dimethylphenoxyaceto-4'-anisidide	ns§	o‡	40
2,4-dimethylphenoxyaceto-4'-chloroanilide	ns§	o‡	20
2,4-dimethylphenoxyaceto-beta-naphthalide	ns§	o‡	0
2,4-dimethylphenoxyaceto-3'-toluidide	ns§	5	−32
2,4-dimethylphenoxyaceto-4'-toluidide	ns§	o‡	22
2-(2',4'-dimethylphenoxyacetamido)-anthraquinone	ns§	o‡	− 5
1-(2',4'-dimethylphenoxyacetamido)-naphthalene-4-sulphonic acid	−33	4	22†
N-2,4-dimethylphenyl N'-2-chlorophenyl urea	ns§	25‡	ns§
N-2,4-dimethylphenyl N'-3-chlorophenyl urea	ns§	o‡	ns§
N-(2,4-dimethylphenyl)-dithiocarbamic acid, ammonium salt	ns§	4	12
2,4-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	o‡	ns§
2,5-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	o‡	ns§
3,4-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	o‡	24
N-2,4-dimethyl-phenyl N'-phenyl urea	ns§	2‡	ns§
3,5-dinitrobenzamide	12¶	o	38†
3,5-dinitrobenzoyl chloride	ns§	25	5
2,4-dinitro-1-naphthol-7-sulphonic acid	17	21	23†
2,4-dinitrophenol	13	9	24†
2,4-dinitrophenoxyacetic acid	21	o	....
N-(2,4-dinitrophenyl)-glycine	13	9	42†
2,4-dinitrophenylhydrazine	ns§	6	18
N,N'-di-(4-nitrophenyl) urea	ns§	6	ns§
N,N-(diphenyl)-2,4-dichlorophenoxyacetamide	ns§	o‡	ns§
N,N-diphenyl N'-phenyl urea	ns§	4‡	ns§
N,N'-diphenylthiourea	ns§	18‡	5
N,N'-diphenyl urea	ns§	o‡	ns§
N,N'-di-(4-sulphamidophenyl)-oxalamide	ns§	o‡	ns§
3,6-disulpho-2-naphthyl N-phenylcarbamate, disodium salt	ns§	8‡	ns§
dithio-oxamide	5	16	25†
ethoxyacetic acid	−29	o	−100
beta-(ethylamino)-ethyl diphenyl-acetate, hydrochloride	−30	o	ns§
ethyl alpha-bromoacetoacetate	−30	13	−75
ethyl 4-bromophenylcarbonate*	−36	26	19§
ethyl 2-bromo-4-phenylphenoxyacetate*	ns§	23‡	57
ethyl 2-secondary-butyl-4-chlorophenoxyacetate*	22‖	o‡	17
ethyl 2-chlorophenoxyacetate*	7	14	−11†
ethyl alpha-(4-chlorophenoxy)-heptoate*	17‖	o‡	−17
ethyl 4-chlorophenylcarbonate*	12	16	29†.
ethyl alpha-cyano-beta-methyl-gamma-ethyl-glutaconate	8‖	o	22†
ethyl 2,4-dibromophenylcarbonate*	ns§	17	31
ethyl dichloroacetate	− 3	12	o†
ethyl 2,4-dichloro-5-aminophenoxyacetate*	25	11‡	− 1†
ethyl 2,4-dichloro-5-nitrophenoxyacetate*	− 8	1	o†
ethyl alpha-(2,4-dichlorophenoxy)-heptoate*	43‖	16‡	−43
ethyl 2,4-dichlorophenylcarbonate*	ns§	35‡	35
ethyl S-(2,5-dichlorophenyl)-thioglycolate	ns§	o‡	−10
ethyl 2-methyl-6-chlorophenoxyacetate	ns§	o	31
2-(3-ethyl-4-methyl-5-cyano-6-hydroxypyridyl)-N-phenylcarbamate	ns§	o	60
2,6-(3-ethyl-4-methyl-5-cyanopyridine)-bis-N-phenylcarbamate	ns§	o	0
ethyl N-(alpha-naphthyl)-carbamate	ns§	o	....
ethyl N-phenylcarbamate	10	o	−15†
bis-beta, beta-ethyl sulphone dilaurate*	ns§	o‡	ns§
ethyl N-(beta,beta,beta-trichloro-alpha-hydroxyethyl)-carbamate	−39	o	13†

TABLE 6—*Continued*

Compound	Test		
	A	B	C
ethyl 2,4,5-trichlorophenoxyacetate	10	0	0†
ethyl S-(2,4,5-trichlorophenyl)-thioglycolate	10	0	0†
4-formylphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
hexachlorophenol	57	18	0‡
hydrazo di-carbonamide	− 1	2	21
4-hydroxyacetanilide	6	0	ns§
4-hydroxybenzaldehyde	−71	0	−111
4-hydroxybenzenesulphonic acid, sodium salt	13	9	ns§
4-hydroxybenzoic acid	−18	31	
O-(2-hydroxybenzoyl)-glycolic acid	− 6	19	9†
2-hydroxy-5-bromobenzoic acid	15	0	19†
2-hydroxy-3-bromo-5-sulphobenzoic acid	−24	39	28†
2-hydroxy-5-chlorobenzoic acid	−13	20	19†
2-hydroxy-5-chloroxenyl mercuric hydroxide	ns§	0‡	ns§
2-hydroxy-3,5-dibromobenzoic acid	− 8	0	16†
2-hydroxy-3,5-diiodobenzoic acid	28	0	−13†
N-(beta-hydroxyethyl) 4-chlorophenoxyacetamide*	9	0	−34†
N-(beta-hydroxyethyl)-dithiocarbamic acid, sodium salt	17	0	ns§
N-(beta-hydroxyethyl) N'-phenyl thiourea	−57	0	−14†
N-(beta-hydroxyethyl)-S-(2,4,5-trichlorophenyl)-thioglycolic amide	10	....	0†
2-hydroxy-3-methyl-5-bromo-benzoyl chloride	ns§	0‡	12
2-hydroxy-3-methyl-5-chloro-benzoyl chloride	ns§	0‡	15
2-hydroxy-4-methyl-6-methoxyquinoline	ns§	0	ns§
2-hydroxy-3-methyl-5-nitrobenzamide	−29	0	ns§
2-hydroxy-3-methyl-5-nitrobenzoyl chloride	1	0	15†
N-(2-hydroxyisopropyl) alpha-(2-methyl-4-chlorophenoxyl)-acetamide*	25	0	−17†
2-hydroxy-4-methylquinoline	− 8	3	ns§
2-hydroxy-3-nitrobenzoic acid	0	0	−17†
2-hydroxy-3-nitro-5-bromobenzoic acid	20	0	42†
2-hydroxy-3-nitro-5-chlorobenzoic acid	−22	17	8†
2-hydroxy-3-nitro-5-sulphobenzoic acid	−25	14	24†
4-hydroxyphenylarsonic acid	− 8	5	ns§
N-(4-hydroxyphenyl)-dithiocarbamic acid, ammonium salt	6	0	−40†
N-(4-hydroxyphenyl)-glycine	− 6	3	6†
2-hydroxypyridine	−14	0	0
beta-hydroxypyridine, sulphate	−22	27	ns§
hydroquinone	−21	0	ns§
5-iodo-2-aminobenzoic acid	2	16	−102†
2-iodobenzoic acid	−33	11	29†
3-iodobenzoic acid	1	29	−70†
2-iodo-3-nitrobenzoic acid*	−34	22	25†
2-iodophenol	17	23	− 9†
N-(4-iodophenyl)-dithiocarbamic acid, ammonium salt	ns§	0	ns§
2-iodophenyl N-phenylcarbamate	ns§	0	44
2-keto-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine	ns§	22	ns§
2-methoxy-4-methylphenoxyaceto-2',5'-dichloroanilide	ns§	0	ns§
2-methoxy-4-methylphenyl N-phenylcarbamate	ns§	0	−28
2-methoxy-4-methylphenyl N-3-tolylcarbamate	ns§	0‡	ns§
2-methoxy-4-methylphenyl N-4-tolylcarbamate	ns§	0‡	ns§
4-methoxyphenylammonium fluoborate	7	22	....
N-(4-methoxyphenyl)-dithiocarbamic acid, ammonium salt	8	0	ns§
4-methoxyphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
N-(4-methoxyphenyl) N'-phenyl urea	ns§	0‡	ns§
2-(methylamino)-benzoic acid	23	0	−15†
beta-methyl-beta-(amylamino)-propyl 4-ethoxybenzoate, hydrochloride	−11	....	31
beta-methyl-beta-(amylamino)-propyl 4-heptyloxybenzoate, hydrochloride	17	22	0†
beta-methyl-beta-(amylamino)-propyl 4-propoxybenzoate, hydrochloride	−20¶	....	....

TABLE 6—*Continued*

Compound	Test		
	A	B	C
beta-methyl-beta-(butylamino)-propyl 4-amyloxybenzoate, hydrochloride	1¶	16	− 6†
beta-methyl-beta-(butylamino)-propyl diphenylacetate, hydrochloride	20¶	o	12
beta-methyl-beta-(butylamino)-propyl 4-heptyloxybenzoate, hydrochloride	8¶	....	19†
2-methyl-6-carboxyphenyl N-(alpha-naphthyl)-carbamate	ns§	o	14
2-methyl-6-chlorophenoxyacetamide	10	o	29†
2-methyl-6-chlorophenoxyacetanilide	ns§	18	30
2-methyl-6-chlorophenoxyacetic (normal-butyl)-amide	ns§	14	7
2-methyl-6-chlorophenoxyaceto-4′-chloroanilide	ns§	o	o
2-methyl-4-chlorophenoxyacetic hydrazide	ns§	27‡	ns§
2-methyl-4-chlorophenoxyaceto-3′-toluidide	ns§	8‡	46
2-methyl-6-chlorophenoxyaceto-3′-toluidide	ns§	o‡	−15
2-methyl-6-chlorophenoxyaceto-4′-toluidide	ns§	7	−14
2-methyl-6-chlorophenoxyacetyl chloride	25	o	25†
alpha-(2-methyl-4-chlorophenoxy)-heptylic acid*	9‖	o	−34
2-methyl-4-chlorophenyl N-phenylcarbamate	− 1	o	−17
2-methyl-5-chlorophenyl N-phenylcarbamate	ns§	o	29
2-methyl-6-chlorophenyl N-phenylcarbamate	ns§	o	24
3-methyl-4-chlorophenyl N-phenylcarbamate	ns§	o	23
2-methyl-4,6-dibromophenoxyacetic acid*	ns§	o	o
2-methyl-4,6-dichlorophenoxyacetic acid*	ns§	15	−41
2-methyl-4,6-dichlorophenoxyacetanilide	ns§	o‡	− 5
2-methyl-4,6-dichlorophenoxyacetic (normal-butyl)-amide	ns§	o	o
2-methyl-4,6-dichlorophenoxyaceto-para-chloroanilide	ns§	5	17
2-methyl-4,6-dichlorophenoxyaceto-2′,5′-dichloroanilide	ns§	14	20
2-methyl-4,6-dichlorophenoxyaceto-3′-toluidide	ns§	o‡	25
2-methyl-4,6-dichlorophenoxyaceto-4′-toluidide	ns§	7	−34
methyl 2-methyl-4,6-dichlorophenoxyacetate	ns§	17	30
beta-methyl-beta-(ethyl-amino)-propyl 4-butoxybenzoate, hydrochloride	−31	....	25†
S-methyl isothiourea sulphate	10	o	ns§
methyl N-(alpha-naphthyl)-carbamate	ns§	o	− 5
2-methyl-1,4-phenylene-bis-(N-alpha-naphthylcarbamate)	ns§	o	ns§
2-methylphenoxyacetamide	28	o	24†
3-methylphenoxyacetamide	−13	13	−10†
4-methylphenoxyacetamide	6	o	24†
3-methylphenoxyacetyl chloride	9‖	26	30†
methyl N-phenylcarbamate	−17	13	46†
N-4-methylphenyl urea	8	o	ns§
beta-methyl-beta-(propylamino)-propyl 4-heptyloxybenzoate, hydrochloride	20¶	....	
4-methyl-4-trichloromethyl 2-chloro-2,5-cyclohexadienone*	−13	o	35†
4-methyl-4-trichloromethyl 2-chloro-2,5-cyclohexadiene-1-one-oxime*	ns§	5‡	8
4-methyl-4-trichloromethyl 2,6-dichloro-2,5-cyclohexadienone*	ns§	12‡	o
4-methyl-4-(trichloromethyl)-5-cyclohexadiene-1-one-oxime*	−16‖	11	25†
4-methyl-4-trichloromethyl-2,5-cyclohexadienone*	22	19	20†
alpha-naphthol	−12	39	o†
beta-naphthol	2	18	39†
1,2-naphthoquinone-4-sulphonic acid, sodium salt	−21	....	
beta-naphthoxyacetamide	ns§	o‡	21
alpha-naphthoxyacetic acid, ammonium salt	− 3	o	o
beta-naphthoxyacetic acid	18	6	25†
2-naphthylamine-6,8-disulphonic acid	− 7	14	ns§
2-naphthylamine-1-sulphonic acid	− 9	o	ns§
1-naphthylamine-4-sulphonic acid	o	9	ns§
1-naphthylamine-8-sulphonic acid	− 7	4	o
N-(alpha-naphthyl)-dithiocarbamic acid, ammonium salt	ns§	o‡	−11
N-(beta-naphthyl)-dithiocarbamic acid, ammonium salt	1	o‡	....

TABLE 6—*Continued*

Compound	Test A	Test B	Test C
N-(alpha-naphthyl)-glycine	−12	1	ns§
alpha-naphthyl N-(alpha-naphthyl)-carbamate	ns§	o‡	ns§
N-(beta-naphthyl) N'-(alpha-naphthyl) urea	ns§	o‡	ns§
N-alpha-naphthyl N'-phenyl urea	ns§	o‡	−14
N-beta-naphthyl N'-phenyl urea	ns§	o‡	−46
N-alpha-naphthyl urea	ns§	o‡	ns§
N,N'-di-(alpha-naphthyl) urea	ns§	26	ns§
nicotinyl chloride-hydrochloride	6	....	10†
nicotinyl nitrile	15	....	20†
4-nitroacetanilide	7	21	ns§
4-nitrobenzamide	7	12	ns§
4-(4'-nitrobenzamido)-benzenesulphonic acid, potassium salt	−6	o	ns§
1-(4'-nitrobenzamido)-2-bromobenzene	ns§	o‡	ns§
1-(4'-nitrobenzamido)-3-bromobenzene	ns§	4‡	ns§
1-(4'-nitrobenzamido)-4-bromobenzene	ns§	o‡	ns§
1-(4'-nitrobenzamido)-2-chlorobenzene	ns§	o‡	ns§
1-(4'-nitrobenzamido)-3-chlorobenzene	ns§	14	−56
1-(4'-nitrobenzamido)-4-chlorobenzene	ns§	6‡	48
1-(4'-nitrobenzamido)-2,5-dichlorobenzene	ns§	o‡	42
1-(4'-nitrobenzamido)-2,4-dimethylbenzene	ns§	o‡	ns§
1-(4'-nitrobenzamido)-4-iodobenzene	ns§	o‡	ns§
1-(4'-nitrobenzamido)-4-nitrobenzene	ns§	o‡	95
3-nitrobenzenediazonium fluoborate	−29	o	ns§
4-nitrobenzenediazonium fluoborate	−15	o	ns§
4-nitrobenzoic acid	37	36	10
3-nitrobenzoic acid	1	14	....
4-nitrobenzyl tris-(hydroxymethyl)-methyl amine	−4	o	ns§
2-nitro-3-bromobenzoic acid*	ns§	o	43
2-nitro-4-bromobenzoic acid*	−21	o	27
3-nitro-4-bromobenzoic acid*	ns§	o	45
2-nitrophenoxyacetic acid	−6	6	47†
4-nitrophenoxyacetic acid	13‖	o	16†
N-2-nitrophenyl N'-2-chlorophenyl urea	ns§	o‡	ns§
N-3-nitrophenyl N'-2-chlorophenyl urea	ns§	o‡	ns§
N-4-nitrophenyl N'-2-chlorophenyl urea	ns§	o‡	−42
N-(4-nitrophenyl)-dithiocarbamic acid. ammonium salt	3	o	27†
N-2-nitrophenyl N'-phenyl urea	ns§	o‡	ns§
N-3-nitrophenyl N'-phenyl urea	ns§	o‡	20
N-4-nitrophenyl N'-phenyl urea	ns§	o‡	ns§
S-(4-nitrophenyl)-thioglycolic acid	−24	4	ns§
5-nitro-2-pyridyloxyacetic acid*	1	27	ns§
4-nitroso-N,N-dimethylaniline, hydrochloride	−48	3	ns§
4-nitrotoluene	−16	o	−23
octadecyl 2,4-dichlorophenoxyacetate*	ns§	69‡	61
normal-octyl carbomethoxymethyl sulphone*	−7	o	34
octyl carboxypropyl sulphide*	1‖	o‡	−10†
oxaloacetic cyclo-aminoguanidide	16	o	ns§
normal octyl 3-methyl-phenoxyacetate	ns§	37	25
penta-chloronaphthalene*	ns§	22	14
penta-chlorophenol	ns§	o‡	−4
penta-chlorophenoxyacetic acid*	ns§	o	ns§
penta-chlorophenyl 3-bromo-3,5-dichlorobenzoate	ns§	26	−15
penta-chlorophenyl alpha-chloroacetate	ns§	o‡	−4
penta-chlorophenyl 2,4-dichlorophenoxyacetate	ns§	93	98
penta-chlorophenyl N-phenylcarbamate	ns§	o‡	34
phenol	5	23	−9†
phenol 2-carboxylic-4-sulphonic acid	−14	o	−29
phenoxyacetamide	−26	o‡	ns§
phenoxyacetanilide	26	7	18†

TABLE 6—Continued

Compound	Test A	Test B	Test C
phenoxyacetic acid	5	13	−14†
phenoxyaceto-4-bromoanilide	ns§	o‡	33
N-phenyl N'-(2-[6-aminopyridyl]) urea	ns§	16‡	ns§
phenylammonium fluoborate	o	o	31
phenyl azide	ns§	o‡	37
N'-phenyl biuret	ns§	o‡	ns§
N-phenylcarbamylguanidine	ns§	10‡	ns§
N-phenyl N'-carboxymethyl urea	ns§	o	ns§
N-phenyl N'-chloroacetyl urea	7	o	35†
N-phenyl-alpha-chloroacetamide	− 6	2	10†
9-phenyl-1,2-7,8-dibenzoxanthene	ns§	26	4
N-phenyl-2,4-diketo-3,4-dihydro-5,6-benzo-1,3-oxazine	ns§	o‡	2†
N-phenyldithiocarbamic acid, ammonium salt	10	7‡	− 9†
1,4-phenylene-bis-(N-alpha-naphthylcarbamate)	ns§	o	ns§
N-phenylglycine	− 8	15	12
N-phenyl N'-(2-hydroxyethyl) urea	−19	19	30†
phenyl isocyanate	−30	o	30†
phenyl isothiocyanate	ns§	o‡	20
N-phenyl N'-alpha-naphthyl urea	ns§	33‡	ns§
2-phenylphenol	20	o	....
4-phenylphenol	−33‖	15‡	....
2-phenylquinoline-4-carboxylic acid	1	o	−19†
N⁴-phenylsemicarbazide	ns§	o	ns§
N-phenyl thiourea	13	o‡	ns§
N-phenyl urea	ns§	5	ns§
potassium ethyl xanthogenate	−10	o	ns§
potassium methyl xanthogenate	7	o	ns§
2-isopropyl-4-chloro-5-methylphenoxyacetic acid	− 2	o	14†
normal-propyl N-(alpha-naphthyl)-carbamate	ns§	o	18
isopropyl N-(alpha-naphthyl)-carbamate	ns§	47	42
N-isopropyl O-phenylcarbamate*	−11	o	9†
normal-propyl N-phenylcarbamate	ns§	15	ns§
pyridine (sulphate)	2	o	42
pyridine-2-carboxylic acid, sodium salt	− 5	o	57†
N-2-pyridyl N'-alpha-naphthyl urea	ns§	o‡	ns§
N-2-pyridyl N'-phenyl urea	ns§	12	ns§
quinoline ethiodide	−13	o	ns§
8-quinolinoxyacetic acid*	5	29	ns§
resorcinol	25	o	o†
sodium anthraquinone-2-sulphonate	− 8	24	ns§
sodium 4-bromophenylsulphamate	5‖	o	ns§
sodium N-cyclohexylsulphamate	16	o	16†
sodium 2,4-dichlorobenzylsulphonate*	− 9	6	2†
sodium phenylsulphamate	− 9	o	ns§
sulphadiazine	3	1	ns§
sulphaguanidine	− 5	o	....
sulphanilamide	24	17	ns§
sulphanilic acid	o	3	....
7-sulpho-2-naphthyl N-phenylcarbamate, sodium salt	ns§	5	ns§
4-sulphophenyl N-(alpha-naphthyl)-carbamate, sodium salt	ns§	o	....
sulphapyridine	14	28	....
tetrabromophthalic acid, sodium salt	−18	28	ns§
N-(2-thiazolyl) N'-(alpha-naphthyl) urea	ns§	23	ns§
2-thioketo-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4,-tetrahydropyrimidine	ns§	30	o
thiourea	6	33	ns§
3-tolyl isocyanate	ns§	3‡	− 5
4-tolyl N-phenylcarbamate	ns§	o	−118
N-2-tolyl urea	ns§	47	ns§

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N-3-tolyl urea	ns§	o	ns§
N,N',di-(4-tolyl) urea	ns§	o	ns§
2,4,6-tribromo-3,5-dimethylphenol	16	o	−34†
alpha, alpha-trichloroacetanilide	22	o‡	− 5†
2,4,5-trichlorobenzenesulphonyl chloride	ns§	o	22
2,2,2-trichloroethyl 2'-methyl-4'-chlorophenoxyacetate	ns§	2‡	31
alpha, alpha, alpha-trichloro-beta-hydroxy-gamma-(3-ethyl-4-pyridyl)-propane	18	18	13
4,x,y-trichloro-2-methylphenoxyacetamide	ns§	6	17
4,x,y-trichloro-2-methylphenoxyacetic acid	−17‖	4	o†
alpha, alpha, alpha-trichloro-beta-hydroxy-gamma-(4-pyridyl)-propane	8	39	ns§
2,4,6-trichlorophenol	11‖	o	25†
2,4,5-trichlorophenoxyacetamide	ns§	48	ns§
2,4,6-trichlorophenoxyacetamide	ns§	19	8
1-(2',4',5'-trichlorophenoxyacetamido)-anthraquinone	ns§	o	−40
2,4,5-trichlorophenoxyacetic N-(2-aminoethyl)-amide	ns§	o	114
2,4,5-trichlorophenoxyaceto-2'-bromoanilide	ns§	o‡	ns§
2,4,5-trichlorophenoxyaceto-2'-chloroanilide	ns§	....	ns§
2,4,5-trichlorophenoxyaceto-4'-chloroanilide	ns§	o	ns§
2,4,5-trichlorophenoxyaceto-2',5'-dichloroanilide	ns§	22	....
2,4,5-trichlorophenoxyaceto-(diphenyl)-amide	ns§	o‡	−13
2,4,5-trichlorophenoxyacetohydrazide	ns§	10‡	ns§
2,4,5-trichlorophenoxyacetic N-(2'-hydroxyethyl)-amide	ns§	13	ns§
2,4,5-trichlorophenoxyaceto-alpha-naphthalide	ns§	o	ns§
2,4,5-trichlorophenoxyaceto-3'-nitroanilide	16	1	9†
2,4,5-trichlorophenoxyaceto-4'-xenylamide	26	o	−53†
2-(2',4',6'-trichlorophenoxyacetamido)-anthraquinone	ns§	o	31
4-(2',4',5'-trichlorophenoxyacetamido)-azobenzene	ns§	o	− 5
4-(2',4',6'-trichlorophenoxyacetamido)-benzene-sulphonic acid	ns§	....	....
2,4,6-trichlorophenoxyacetyl chloride	ns§	17	15
N-(2,4,6-trichlorophenyl)-alpha-chloroacetamide	ns§	6‡	35
N-2,4,6-trichlorophenyl N'-phenyl urea	ns§	o	ns§
2,4,6-trichlorophenylsulphamic acid (sodium salt)	12	o	24†
S-(2,4,5-trichlorophenyl)-thioglycolic acid	17	12	− 5†
S-(2,4,5-trichlorophenyl)-thioglycolic chloride	17	o‡	13†
S-(2,4,5-trichlorophenyl)-thioglycolic anilide	ns§	1‡	12
S-(2,4,5-trichlorophenyl)-thioglycolic 2'-bromoanilide	ns§	o‡	29
S-(2,4,5-trichlorophenyl)-thioglycolic 3'-bromoanilide	ns§	o‡	12
S-(2,4,5-trichlorophenyl)-thioglycolic 4'-bromoanilide	ns§	o	12
S-(2,4,5-trichlorophenyl)-thioglycolic (normal-butyl)-amide	ns§	8‡	o
S-(2,4,5-trichlorophenyl)-thioglycolic 3'-chloroanilide	ns§	o‡	6
S-(2,4,5-trichlorophenyl)-thioglycolic 2',4'-dichloroanilide	ns§	17‡	o
S-(2,4,5-trichlorophenyl)-thioglycolic diphenylamide	ns§	o‡	8
S-(2,4,5-trichlorophenyl)-thioglycolic 3'-toluidide	ns§	12‡	o
2,4,5-trichlorothiophenol	ns§	o‡	− 8
tricresyl phosphate	10	8‡	11†
triethyl phosphate	o	8	o†
N-(3-[trifluoromethyl]-phenyl)-alpha-(2-methyl-4-chlorophenoxy)-acetamide*	ns§	23‡	37
2,3,5-triiodobenzamide	ns§	o	ns§
2,4,5-triiodobenzoic acid*	ns§	o	40
2,4,6-trinitrophenyl N-phenylcarbamate	24	11	−11
triphenyl phosphate	ns§	11	o
N-(tris-[hydroxymethyl]-methyl)-morpholine, hydrochloride	−12	o	ns§
2-xenylammonium fluoborate	−16	o	ns§
4-xenylammonium fluoborate	9	32	....
2-xenyloxyacetamide	17¶	o	19†
2-(2'-xenyloxyacetamido)-anthraquinone	ns§	o‡	8
2-xenyloxyaceto-3-xenylamide	ns§	o‡	37

TABLE 6—*Continued*

COMPOUND	TEST		
	A	B	C
2-xenyloxyacetyl chloride	1 2	0	19†
N-2-xenyl N'-2-chlorophenyl urea	ns§	40‡	ns§
N-2-xenyl N'-3-chlorophenyl urea	ns§	58‡	−30
N-4-xenyl N'-2-chlorophenyl urea	ns§	0‡	7
N-4-xenyl N'-3-chlorophenyl urea	ns§	0‡	− 2
N-4-xenyldithiocarbamic acid, ammonium salt	ns§	0‡	ns§
N-2-xenyl N'-phenyl urea	ns§	0‡	45
N-4-xenyl N'-phenyl urea	ns§	0‡	ns§

A SIMPLE BIO-ASSAY METHOD FOR THE DETERMINATION OF LOW CONCENTRATIONS OF 2,4-DICHLOROPHENOXY-ACETIC ACID IN AQUEOUS SOLUTIONS[1]

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Introduction

In the course of various studies relating to the movement and persistence of 2,4-dichlorophenoxyacetic acid in different soil types and to the uptake of this growth-regulating substance from nutrient solution by the roots of broadleaf and cereal plants, the need arose for a simple bio-assay method which would accurately test for unknown concentrations of the acid in aqueous solutions. Colorimetric methods had to be discarded because no colored derivatives or degradation products could be readily formed. Various methods were tried, in which stem curvature of seedling plants and formative effects on kidney-bean and tomato plants were used as criteria; but it was soon apparent that, while these readily indicated the effects produced by low concentrations of the com-

pound, they did not give sufficiently critical evaluations of its action.

The success attained in determining the growth-inhibitory effectiveness of certain chemicals by the corn-germination test (1) suggested that the degree of elongation of the primary root of corn seedlings might possibly be used as a criterion. In this test it was shown that low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solution had a marked inhibitory effect on root elongation of germinating corn. With certain modifications, this method met the requirements demanded of it, and the procedure and results are described below.

Material and methods

The seed material used throughout this study and for all later bio-assay determinations was a hybrid field corn, var. Silver King (Wisconsin no. 7).

The unselected kernels were first sterilized in a saturated solution of sodium

hypochlorite for 3 minutes, after which they were placed in 6-inch Petri dishes on filter paper moistened with distilled water. Twenty to twenty-five kernels were placed in each dish, with the embryos facing downward. They were then allowed to germinate and grow in a dark-

TABLE 1

ROOT GROWTH, COMPUTED IN PERCENTAGE OF CONTROL, OF GERMINATING CORN SEEDS SUBJECTED TO VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN AQUEOUS SOLUTION. EACH FIGURE IS BASED ON APPROXIMATELY SEVENTY-FIVE MEASUREMENTS

Concentration (p.p.m.)	Root growth (%)	Concentration (p.p.m.)	Root growth (%)
0.001	95.8	2.5	18.5
0.005	97.0	3.0	16.0
0.01	96.0	3.0	13.9
0.01	97.0	4.0	11.2
0.1	81.6	4.0	10.6
0.1	78.2	4.0	13.2
0.2	73.0	4.5	11.2
0.2	75.1	5.0	11.0
0.3	65.0	5.0	9.2
0.4	57.3	5.0	10.5
0.4	58.0	6.0	11.5
0.5	53.1	6.0	10.8
0.5	53.0	7.0	10.0
0.5	52.6	7.0	8.8
0.75	37.8	7.5	6.4
1.0	35.3	8.0	10.1
1.0	33.7	8.0	8.9
1.0	32.1	9.0	14.2
1.5	25.8	9.0	9.4
1.5	27.2	10.0	9.8
2.0	22.0	10.0	8.9
2.0	20.5		

room at 26° C. for 48 hours. The dishes were removed from the darkroom at the end of this period, and those seeds, the primary roots of which had attained a length of 15–25 mm., were removed, measured, and placed in other 6-inch Petri dishes on filter paper moistened with 15 ml. of solutions containing various concentrations of 2,4-dichlorophenoxyacetic acid. Twelve to fifteen

seeds were placed in each dish, and a record was kept of the average root lengths of the seeds (approximately seventy-five in number) used for each of the concentrations tested. After 48 hours of further growth in the darkroom, the seeds were removed, and the primary roots were again measured. By subtracting the average root length obtained at the time of the first measurement from the average root length obtained at the time of the second measurement, the amount of root elongation which oc-
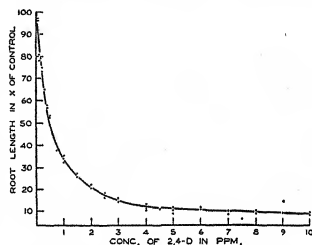
FIG. 1.—Degree of primary root elongation of germinated corn seeds when exposed for 48 hours to various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-d).

curred during the 48 hours that the seeds were in contact with the 2,4-dichlorophenoxyacetic acid solutions could be determined. The untreated series were similarly handled, with the exception that distilled water was used throughout. Solutions of unknown concentration were handled in a similar manner, with dilutions being made whenever necessary.

Observations

The results of the numerous trials with concentrations ranging from 1/1000 p.p.m. to 10 p.p.m. are presented in table 1. The data are graphically presented in figure 1, the amount of root elongation being computed as percentage of control.

With this curve used as a basis of comparison for the determination of unknown concentrations of 2,4-dichlorophenoxyacetic acid, it is evident that the greatest accuracy will be obtained for concentrations ranging from 1/100 to 2 p.p.m. A reasonable degree of accuracy may be obtained for concentrations between 2.0 and 4.0 p.p.m.; but, as the concentrations increase, the accuracy of determination decreases. Dilutions of unknown concentrations, however, can readily be made to the more accurately tested lower levels; and repeated tests of solutions the concentrations of which were unknown to the author but known to other members of these laboratories reveal that the concentration of such solutions may be determined within the limits of a 5 per cent error.

The accuracy of the corn-germination test, especially when large samples are used, suggests that this bio-assay method may be of value in the determination of unknown concentrations of other growth-regulating substances, provided that similar reference curves are developed for the substance in question.

Summary

1. A simple method for the bio-assay of 2,4-dichlorophenoxyacetic acid in aqueous solution is described, whereby the activity of the compound is measured by its ability to inhibit the elongation of the primary root of germinating corn seed.

2. Unknown concentrations of the compound, ranging from 1/100 to 2 p.p.m., can be accurately determined by this method; it is less accurate for amounts between 2 and 4 p.p.m. and is suitable only for preliminary testing in the range of 4–10 p.p.m. and above.

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ABSORPTION AND TRANSLOCATION OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

The growth-regulating substance, 2,4-dichlorophenoxyacetic acid, produces pronounced morphological responses in plants at a considerable distance from the point of application (3). Such effects have been termed telemorphic by BEAL (1). Stem curvature, epinasty, prolifera-

tion of various plant parts, and formation of gall-like growths are common responses to single droplet or spray applications of this compound. Since the acid can be detected in extremely small amounts, a study of its translocation in plants is not difficult.

HITCHCOCK and ZIMMERMAN (3) have demonstrated that synthetic growth substances can be absorbed from the soil by plants and transported upward, as are

mineral salts. FERRI (2) recently studied translocation of such substances by applying the compounds to intact plants, either as solutions poured into the soil or as lanolin preparations applied around the stem. He showed that upward movement of certain regulators was intense enough to induce rooting of leaf cuttings taken from tomato and *Cleome* and planted in sand at a certain interval after treatment. He observed no movement of growth regulators on leafless *Hibiscus* cuttings when a segment of wood was removed.

The results of the experiments presented in this paper support the theory of upward movement of growth regulators in the xylem and lend evidence to the view that the phloem is the important tissue involved in downward movement.

Experimentation

Absorption of the growth regulator by plant tops

When 2,4-dichlorophenoxyacetic acid is brought into contact with aerial portions of plants, it presumably enters by penetration of the cuticle, epidermal layer, and underlying cells. An experiment was conducted which indicates that stomata may be unimportant as a portal of entry for the growth regulator. Young plants of *Nasturtium* and *Coleus* were selected for experimentation as they have stomata on only the lower surfaces of the leaves. Using a 200-ml. pressure pump, the plants were sprayed with a 0.1% solution of the ammonium salt of the acid in such a manner that in some plants only the lower surface and in others only the upper surface of the leaves was exposed to the spray. Tests were conducted on *Nasturtium* (three pots of three plants each) and on *Coleus* (two pots of one plant each). At time of harvest, 23 days

after treatment, the fresh weight of leaf blades was determined, as this has been shown to be an excellent criterion in a study of growth inhibition of plants caused by this growth regulator (4). The results indicated that, regardless of the particular leaf surface sprayed, the degree of inhibition was about the same. However, when plants are exposed to volatile compounds or to an aerosol, stomata probably play a more important role.

The bark of stems appears to retard entry of the growth regulator into the transpiration stream (2). One drop of an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid was placed on cotyledons of young plants of soybean, the upper surfaces of some cotyledons being scratched with sandpaper before application. Those plants whose cotyledons were scratched were the only ones that developed curvatures, and, furthermore, they showed the least recovery. Scratching the cotyledons may have exposed some xylem tissue which may have permitted rapid entrance of the growth regulator into the plant.

Similar experiments were performed in which the compound was applied dropwise to scratched and uninjured leaves of red kidney bean. In these tests the rupturing of the leaf tissue did not aid entry of the agent into the plant. The compound entered the main portion of the plant by moving basipetally in living tissue, presumably the phloem. The cuticle and epidermal layers did not retard entrance of the compound into the leaf.

2,4-Dichlorophenoxyacetic acid often enters the leaf with great rapidity. On warm sunny days, young broadleaf plants sprayed with this compound may exhibit epinasty and stem curvature within 1 hour after treatment, indicating rapid absorption and translocation. In

another experiment droplets of an aqueous solution of the compound were placed on the base of blades of primary leaves of young soybean plants by means of a hypodermic syringe. At intervals from 1 to 24 hours after treatment the blades were snipped off just below the point of application, and growth of the plants was observed for 21 days. It was concluded that the maximum amount of the compound entered the leaf within 6 hours after treatment. A similar conclusion was drawn from experiments in which young red kidney bean plants sprayed with the compound were exposed to artificial rainfall at various intervals after spraying (5). Almost maximum damage ensued if young plants were not subjected to an artificial rainfall until 6 hours after exposure, indicating maximum entry of the compound into the plant within this period.

TRANSPORT THROUGH STEM SEGMENTS OF DEAD TISSUE

Soybeans at two different stages (second trifoliate leaf stage and flowering), red kidney beans at three stages (primary leaf, first trifoliate, and third trifoliate leaf), and cowpeas having three trifoliate leaves were used for experimentation. Three soybeans or cowpeas and two beans were grown in 4-inch pots. Each treatment consisted of two pots. Portions of stems about 1 inch long near the cotyledonary nodes were killed by flaming briefly with an alcohol lamp flame. After several hours the dead segments were bleached a brown or white color, and the dead portions of stems of the tender young beans and soybeans shriveled up. Otherwise the plants appeared uninjured for several days, as water uptake continued through the dead tissues of the xylem. Upward movement of the growth regulator was studied by

adding to the soil 36 ml. of an aqueous solution of the ammonium salt of the acid containing 1 mg. per ml. Twenty-four hours later all plants treated with this compound showed stem curvature. The amount of curvature produced did not differ in uninjured plants and plants having a dead segment in the stem, indicating that the growth regulator readily moved upward in the transpiration stream, or xylem.

Downward movement of the stimulus produced by this compound was studied by applying a small amount of a 1% mixture in lanolin to the stem just above the dead portion. In no instance did the growth regulator move downward through the dead segment of stem (figs. 1, 2). However, this experiment does not provide unequivocal proof of lack of downward movement in the xylem of the uninjured plants, as normal functioning of the xylem was affected when stems were flamed.

TRANSPORT IN DEFOLIATED AND UNINJURED PLANTS

Red kidney beans at three stages (primary leaf, second trifoliate leaf, and flowering), and soybeans at two stages (second and third trifoliate leaf) were used. Three soybeans or two kidney beans were grown in 4-inch pots, and each test was on two pots. Some plants were defoliated at 10.30 A.M. At 3.30 P.M. on the same day both normal and defoliated plants were treated just below the second node with a small amount of a mixture of 1% of the growth regulator in lanolin. It was assumed that in uninjured plants there would be a basipetal movement of carbohydrates in the phloem, but that this flow might be much less in defoliated plants. Twenty-four hours after treatment, the bean plants in primary leaf stage showed stem
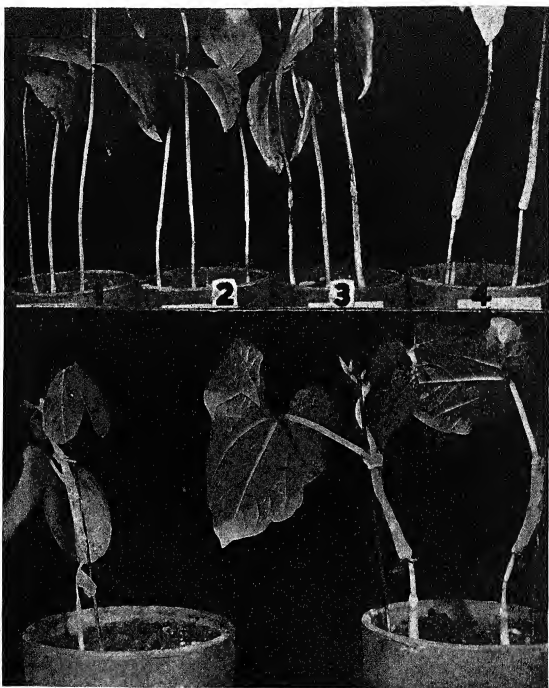
FIGS. 1, 2.—Fig. 1 (above), cowpeas 9 days after treatment with 2,4-dichlorophenoxyacetic acid. 1, control; 2, untreated but with dead stem segment; 3, compound applied to first internode of uninjured plant; 4, compound applied above dead stem segment. Note swelling of first internode and hypocotyl of 3, but that stem below dead segment in 4 is unaffected. Fig. 2 (below), soybean (left) and red kidney bean (right) several days after 2,4-dichlorophenoxyacetic acid was applied to stem above dead stem segment. Note great enlargement of stem except below dead segment.
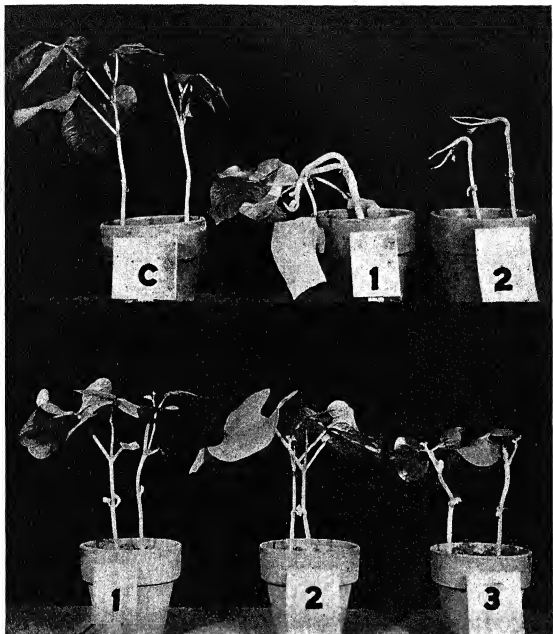
FIGS. 3, 4.—Fig. 3 (above), red kidney beans 6 days after 2,4-dichlorophenoxyacetic acid was applied to the stem below the second node. *C*, normal and defoliated controls. Plants with leaves, 1, have greatly thickened hypocotyl and first internode, and stem curvature occurred several inches below application of the compound. Defoliated plants, 2, have no thickened stem, and angle of stem curvature is sharp and near application of the compound. Fig. 4 (below), red kidney beans 5 days after treatment. On control, 1, note one primary leaf has been largely removed by clipping. The ammonium salt of 2,4-dichlorophenoxyacetic acid applied to clipped leaf, 2, caused no growth inhibition; but the compound applied to unclipped leaf, 3, resulted in severe stunting of plants.

curvature (fig. 3). When the application was made to young uninjured plants, stem curvature occurred several inches below the point of application, but with defoliated plants there was only a sharp-angle bend in the stem adjacent to the point of application. This indicated that there was probably little or no downward movement of the compound in defoliated plants.

When final observations were made 8 days after treatment, it was evident that stem thickening and gall formation occurred below the point of application in the case of uninjured plants. However, no such responses depending on downward movement of the compound were manifested in defoliated plants (fig. 3).

TRANSPORT IN PLANTS HAVING PARTIALLY CLIPPED LEAVES

Red kidney bean plants were grown in 4-inch pots, and at time of treatment they had expanded their primary leaves. Four pots were used for one test. One primary leaf of some plants was partially removed so that only about a $\frac{1}{4}$ square inch of the blade remained. The effect of such clipping, by removing actively photosynthetic leaf tissue, would be to reduce the quantity of elaborated carbohydrate material passing downward through the phloem tissues. Twelve hours after leaves were clipped, 0.02 ml. of an aqueous solution containing 10 gamma of the ammonium salt of 2,4-dichlorophenoxyacetic acid was applied to the clipped primary leaves of some plants and to the unclipped leaves of others. The liquid was applied to the bases of the blades with a hypodermic syringe. Since little plant response occurred, another 20 gamma of compound was applied in the same manner and at the same places 24 hours after the first treatment. Six hours after the second application, the stems of

seven of the eight plants that received the compound on the unclipped leaf showed curvature, and the average curvature was 54 degrees. No other plants exhibited stem curvature.

Plants receiving the compound on clipped leaves made wholly normal growth, but those receiving it on unclipped leaves were severely inhibited. Plants were harvested 12 days after treatment. The average fresh weights on a two-plant basis of trifoliate leaf blades of untreated control plants, untreated plants with clipped leaves, plants receiving treatment on the clipped leaf, and plants receiving treatment on the unclipped leaf were 5.22, 5.28, 4.96, and 1.71 gm., respectively (fig. 4). The least difference for significance between treatment means at the 1% and 5% levels was 1.17 and 0.81 gm., respectively. It is reasonable to assume that much more carbohydrate was being produced in the unclipped than in the clipped leaf, owing to the presence of greater leaf surface. Translocation of the growth regulator seems to accompany the presence of synthesized material.

TRANSPORT FROM VARIOUS PORTIONS OF PRIMARY LEAVES

Red kidney bean plants were grown in 4-inch pots and treated when the primary leaves were expanding. Four gamma of compound in 0.02 ml. of aqueous solution was applied with a hypodermic syringe to the base, middle, or tips of the upper surfaces of one primary leaf. Sixteen days after treatment, the fresh weights of plants above the primary node on a two-plant basis were 10.10, 6.09, 3.88, and 2.88 gm., respectively (fig. 5).

EFFECT OF LIGHT ON TRANSPORT

It has frequently been observed that the curvature responses produced by the
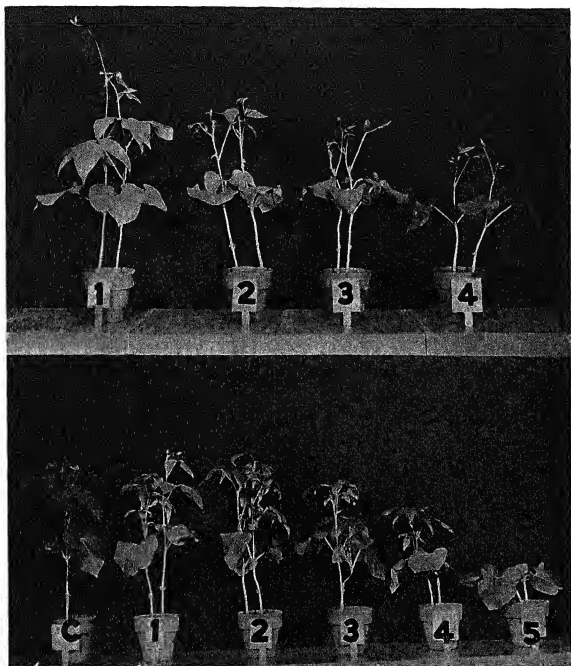
FIGS. 5, 6.—Fig. 5 (above), red kidney beans 16 days after treatment with 4 gamma of 2,4-dichloro-phenoxyacetic acid at tip (2), middle (3), and base (4) of the blade of one of the primary leaves. Control at left. All growth above primary leaves was produced after treatment. Most growth inhibition resulted when the compound was added to base of blade (4). Fig. 6 (below), red kidney beans 17 days after plants were treated with 10 gamma of the ammonium salt of 2,4-dichlorophenoxyacetic acid placed near the tip of one of the primary leaves of each plant. Control at left. The compound was removed from plants in 1, 2, 3, and 4 by removing the treated portions of the leaves 5, 8, 12, and 24 hours after treatment, respectively. Treated portions of plants in 5 were not removed. Much stunting of growth resulted when the compound remained on plant 24 hours or more.

application of 2,4-dichlorophenoxyacetic acid are less rapid and less pronounced if cloudy rather than sunny weather prevails after treatment. This phenomenon is probably related to rate and quantity of transport of carbohydrate as previously discussed. In full sun, photosynthesis may proceed actively, and both downward and upward movements in the plants would be greatly influenced by the relative conditions of light and shade prevailing.

Red kidney bean plants were grown, two per 4-inch pot, and at the time of

TABLE 1

AVERAGE CURVATURE (IN DEGREES) OF STEMS OF PLANTS GROWING IN SUNLIGHT AND SHADE

	TIME INTERVAL AFTER TREATMENT		
	4 hours	24 hours	28 hours
Shade..............	0	4	9
Sunlight...........	15	30	36

treatment were expanding their primary leaves. At 11.00 A.M. on a sunny day some plants were placed under a small lattice shelter covered with three thicknesses of newspaper. At 8:30 A.M. on the following day, sets of plants in sunlight and in shade were treated dropwise at bases of one leaf blade with 10 gamma of the compound in 0.02 ml. aqueous solution. Since no stem curvature occurred, another 20 gamma of compound was similarly applied 3 hours later. At 9:00 A.M. on the day following treatment, the shaded plants were placed in the sun. Each treatment included four pots.

The amount of stem curvature was measured with a protractor. Data in table 1 indicate that most stem curvature occurred in plants growing in the

light. Twenty-four hours after treatment, all treated plants in light showed curvature.

At the time of harvest, 12 days after treatment, controls had three trifoliate leaves. Average fresh weights of trifoliate leaf blades on a two-plant basis for controls in sunlight, controls in shade, treated plants in sunlight, and treated plants subjected to shade were 5.66, 5.82, 1.94, and 3.23 gm., respectively. The least differences for significance of treated means at 5 and 1% were 0.54 and 0.78 gm., respectively. Treated plants in shade produced significantly less weight of trifoliate leaf blades than controls, and treated plants in sunlight produced significantly less than treated plants in shade.

An interesting observation was that the treated leaves of plants in the dark curved upward so that the leaf tip was often over the pulvinus. Under conditions of darkness food materials are often transferred in an upward direction from the cotyledons to the leaves. The fact that the growth regulator seems to move readily in the direction of movement of elaborated food materials perhaps explains the recurving of the leaf.

This experiment indicates that movement of the compound is either more rapid or occurs in greater amount, or both, when rapid movement of synthesized carbohydrate occurs. There is indication that more ultimate injury is done when there is rapid translocation of the compound.

SPEED OF TRANSLOCATION

It has been shown that the speed of upward movement of growth substance is proportional to the transpiration rate of the plant (3). This supports the theory that solutes rise in the transpiration stream of the xylem. The velocity of movement of certain growth regulators

is influenced by the photosynthetic activity of the plant, age of the tissue involved, and several other factors.

An experiment was performed to determine the speed of transport of the growth regulator in the primary leaf of the kidney bean. Plants were grown two per 4-inch pot and treated when the primary leaves were expanding. Each treatment included three pots. Aqueous drops of 0.04 ml. containing 40 gamma of the ammonium salt of the acid were placed on the upper surface of the primary leaf near the center of the leaf. The plants were treated at 9:00 A.M. on a clear sunny day. At intervals of 1 to 72 hours after application, the leaves were snipped close to the plant stem. The distance from the center of the leaf to the base of the petiole was about $1\frac{3}{4}$ inches. Ten days after treatment the plants were harvested and average fresh weights of trifoliate leaf blades obtained. Figure 7 indicates that treated leaves had to remain attached to the plants for over a 24-hour period for attainment of maximum growth inhibition. The amount of such inhibition is proportional to the quantity of growth regulator transported from the treated leaf into the plant stem. Leaves of untreated plants were snipped off at several intervals after treatment, but this had little effect on subsequent growth.

A similar experiment was performed in which drops containing 10 gamma of the compound were placed on the primary leaf of kidney beans toward the tip of the leaf. At intervals of from $\frac{1}{2}$ to 24 hours after application, the leaves were snipped at one-third the distance from the base, thereby removing the treated area. In general, the results of this experiment were similar to those described in the preceding paragraph (fig. 6).

Regeneration studies were performed

to determine whether prompt cutting back or clipping of a crop after spraying with a growth regulator would prevent or reduce the extent of the injury that might otherwise be caused. The results would be dependent on the speed at which the substance is transported from the upper to the lower plant parts. Alfalfa was grown in 6-inch pots, and at time of treatment the plants had an average
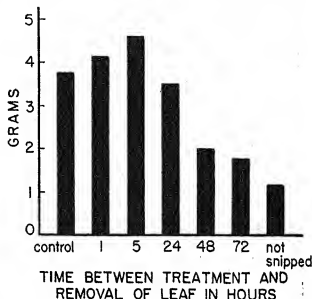
FIG. 7.—Average fresh weights on a two-plant basis of trifoliate leaf blades of red kidney bean, the primary leaves of which were treated with the ammonium salt of 2,4-dichlorophenoxyacetic acid and then removed after various intervals.

foliage height of 12 inches. Each treatment included two pots. Plants were sprayed with an aqueous solution of the ammonium salt of the acid at a rate of 100 mg. in 10 ml. per square yard of ground surface. Plants were then clipped at a height of 2 inches at intervals of 1, 6, 13, 24, and 48 hours after treatment. Unsprayed controls were clipped, and one set of sprayed plants was not clipped. At time of harvest, 27 days later, the sprayed unclipped plants were almost dead, and the unsprayed clipped plants had made much new growth (fig. 8). Regeneration of plants clipped 1 hour after treatment was considerable, although the

green weight was less than half that of the clipped but unsprayed control. Only a few stems of plants clipped 6 hours after spraying sent up new growth, and almost no growth occurred if the interval between spraying and clipping was 13 hours or more. At harvest all plants were clipped at a height of 2 inches and the fresh weights on a two-pot basis determined. The weights for untreated controls, for unsprayed plants clipped at the

to, and cotton. Beginning with the lowest leaves, aqueous droplets containing 5 or 25 gamma of compound were placed on the first, second, and third leaves of replicate plants. Sixteen days after treatment, untreated soybean plants, plants receiving compound on the first leaf, on the second leaf, and on the third leaf had heights of 60, 60, 40, and 30 cm., respectively (fig. 9); for tomatoes the heights were 32, 35, 27, and 27 cm. These

Fig. 8.—Alfalfa 27 days after being sprayed with ammonium salt of 2,4-dichlorophenoxyacetic acid. C, control; 1, control, clipped; 2, treated, clipped after 1 hour; 3, treated, clipped after 6 hours; 4, treated, clipped after 13 hours; 5, treated, clipped after 24 hours; 6, treated, not clipped.

beginning of the experiment, and for plants sprayed and clipped 1, 6, 13, 24, and 48 hours after treatment were 38.1, 22.5, 10.8, 3.4, 2.0, 00, and 00 gm., respectively. The weight of plants treated but not clipped was 10.4 gm. The results indicate that there is a rapid downward transport of the growth regulator in alfalfa.

INFLUENCE OF AGE OF TISSUES ON ABSORPTION AND TRANSLOCATION

An experiment was performed in which equal applications of 2,4-dichlorophenoxyacetic acid were made to successive leaves on young plants of soybean, toma-

results indicate that the younger the leaf the greater the ultimate inhibition of growth of the plants, when plant stature is used as a measure of growth. There is as yet no satisfactory explanation of this observation. Proximity to the apical meristem, ease of entrance into or of absorption by the leaf, rapidity or readiness of transport of the compound or its effects, all may be involved.

In a second experiment which yielded similar results 100 gamma of the compound were applied to the upper or second trifoliate leaf of soybeans, or to the lower or primary leaf. Seventeen days after treatment, plants, the upper leaves of
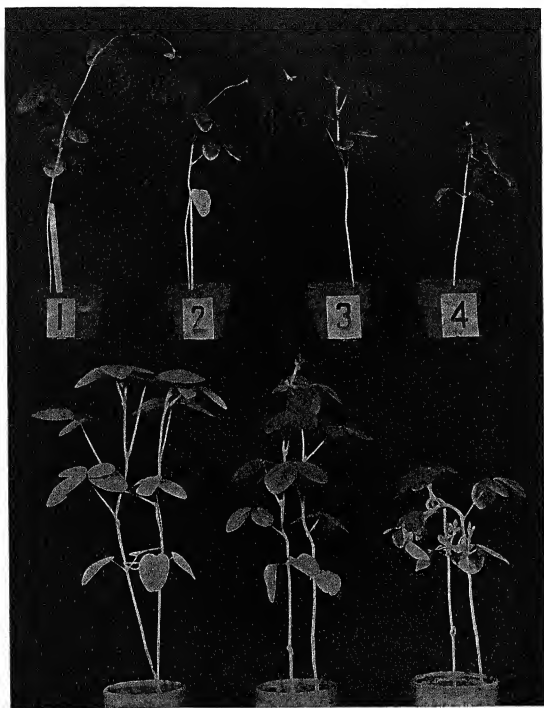
FIGS. 9, 10.—Fig. 9 (above), soybeans received 25 gamma of 2,4-dichlorophenoxyacetic acid as a droplet on primary leaf, 2, second leaf, 3, and third leaf, 4. Control is at left. Treatment of younger leaves caused more stunting of growth. Fig. 10 (below), soybeans 17 days after 100 gamma of 2,4-dichlorophenoxyacetic acid was added to primary leaves (middle), and second trifoliate leaves (right). Control at left. Note that when second trifoliate leaf was treated, plant was stunted and produced axillary shoot. Plants whose primary leaves were treated had enlarged hypocotyls and first internodes.

which were treated, showed severe stunting (fig. 10). Stems were bent, and no flowers had formed. Axillary shoots 1–5 inches long had grown from the cotyledonary or the second node. Leaves had a deep green color, and gall-like calluses had formed along veins of treated leaves, especially near the basal portions. Plants whose lower leaves had been treated were flowering and appeared normal, except that young leaves were deformed. The

evidenced by gall formation and stem thickening. When the hypocotyl of a young kidney bean plant was treated, the swelling of the stem was usually limited to the hypocotyl, but treatment of the first internode produced stem thickening both in hypocotyl and in first internode (fig. 11). Leaves which expanded after plants were treated showed typical abnormalities induced by the agent and were evidence of subsequent upward
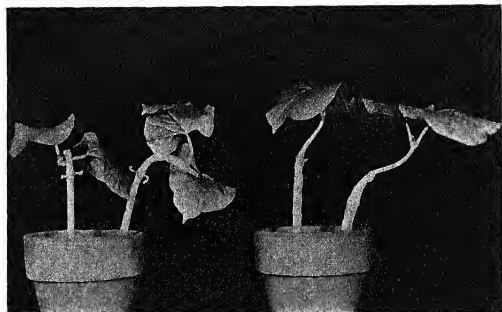
FIG. 11.—2,4-dichlorophenoxyacetic acid applied to first internode of red kidney bean (left) caused both first internode and hypocotyl to enlarge. Treatment of hypocotyl (right) had little effect on first internode.

hypocotyl and first internode showed much more swelling than the corresponding parts of the other plants. Average fresh weights of leaf blades for plants whose upper and lower leaves were treated on a two-plant basis were 3.75 and 4.46 gm., respectively.

Other experiments were carried out in which applications of the compound in lanolin were made to various portions of stems of kidney bean, tomato, cowpea, and soybean, and the subsequent effects, inhibitory and formative, noted. In the kidney bean plants the movement of the agent seemed to be chiefly downward, as

movement of the compound. In general, when lower portions of stems were treated, the compound seemed to move downward and a minimum over-all stunting of plant growth occurred. When upper portions of stems were treated, apical growth was inhibited, much over-all stunting of plants occurred, and axillary shoots often developed.

Summary

1. The pronounced morphological response in plants to 2,4-dichlorophenoxyacetic acid is useful in studies of translocation.

2. The regulator passed upward but not downward through dead segments of stems.

3. Stomata did not appear to be important portals for entry of the compound into the leaves when it was used in aqueous sprays. Leaves of young soybeans absorbed maximum amounts of the growth regulator within 6 hours after application.

4. The movement of food materials was regulated by partial clipping of leaves, defoliation, and reduced light intensity. When the growth regulator was applied to aerial portions of plants, the substance readily moved downward whenever translocation of synthesized materials occurred.

5. More extensive growth inhibition of young red kidney bean plants at primary leaf stage resulted when the substance was added to the base of the blade than when added near the tip. When the compound was added to a primary leaf of a young bean plant more extensive growth inhibition resulted when translocation of the growth substance was rapid than when it was slow.

6. When the growth regulator was added to the primary leaf of kidney beans, it was rapidly translocated basipetally to the stem. In one experiment leaves treated with 40 gamma of the growth regulator had to remain on the plant for 24 hours for maximum amount of compound to reach the stem. Experiments in which alfalfa was sprayed with the growth regulator and then clipped at subsequent intervals showed that much of the compound was translocated from upper to lower plant parts within 1 hour. The velocity of downward movement is markedly influenced by the photosynthetic activity of the plant and the age of the tissue involved.

7. When upper portions of stems or of younger leaves were treated with the compound, apical growth was inhibited, much over-all stunting of plants occurred, and axillary shoots often developed. When lower portions of stems or of older leaves were treated much less stunting of plants occurred. There is as yet no explanation for this occurrence. Proximity to the apical meristem, ease of entrance into the leaf, rapidity or readiness of transport of the compound—all may be involved.

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HISTOLOGICAL RESPONSES OF THE KIDNEY BEAN TO AQUEOUS SPRAYS OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

Recent interest in the herbicidal activity of phenoxyacetic acids and in their physiological similarity to other growth-regulating substances suggested that a histological study of the responses produced might throw some light on the manner by which these compounds affect various plant tissues. The compound 2,4-dichlorophenoxyacetic acid was selected for study because of its effectiveness at very low concentrations and because, unlike certain other growth-regulating substances studied, its action is systemic rather than local in nature.

Material and methods

The kidney beans used in this study had their primary leaves fully expanded at the time of treatment, and the second internodes had attained lengths of 5–10 cm. The 2,4-dichlorophenoxyacetic acid was applied in the form of a 0.1% aqueous spray with an atomizer at a rate of 30 ml. per square yard (a rather heavy rate of application). This constitutes a lethal dose for kidney-bean plants at this stage of development, but the plants survived sufficiently long to yield satisfactory material for histological study. Following treatment, the plants were grown under normal greenhouse conditions, and collection of material for fixation was made at regular intervals. The collected material was fixed in a formalin-acetic acid-alcohol solution and passed through the butyl alcohol series into paraffin. All

sections were cut at 12μ and stained according to the safranin-fast green schedule.

Gross responses

The responses of the young kidney bean to 2,4-dichlorophenoxyacetic acid are striking. Within an hour or two after the spray treatment, a sharp bending of the first internode takes place (this response is most rapid in bright sunlight). The second internode may likewise bend or twist, and a severe epinasty usually follows. It is evident that a differential elongation of cells must take place to produce these curvatures. The plants have the appearance of being severely wilted, but in no instance was there an indication of wilt, the stems and foliage being noticeably more turgid than untreated material. The plant, as a rule, does not later regain an upright position but retains its curvature permanently.

Within 24 hours a yellowing of the younger portions of the stem occurs, particularly along the rigid portions of the second internodes. This yellowing, due presumably to a loss of chlorophyll and, to some extent, of plastids, increases in extent with the passage of time. The primary leaves, however, remain green and may even take on a darker hue. After 48 hours a noticeable thickening of the second internode becomes evident. This thickening may take various forms. If the second internode is 5 cm. or less, the entire region swells evenly and finally becomes an elongated gall, through which protuberances of various shapes and sizes emerge. Longer internodes, on the other hand, are more likely to form

localized galls at the tip and base. The axillary buds, too, enlarge in size and develop into galls tipped by tiny leaves, which do not increase in size.

The first internode and the hypocotyl are slower to respond, but each takes on a characteristic appearance. The internode, which bends just below the primary leaf node, becomes distinctly ridged, almost corrugated; increases gradually in girth throughout its entire length; and then bursts its cortex as the internally formed root primordia push outward. The hypocotyl, which may or may not increase in diameter, reacts by forming four vertical rows of root primordia, giving the stem a somewhat squared appearance in cross-section. These rows of root initials, when followed downward, merge with the tetrarch root system underground.

The formation of roots in considerable profusion on the stem, petioles, and even the midribs of leaves is one of the most characteristic of the responses. Generally, root primordia are formed only on the stem; but, where heavy local applications of 2,4-dichlorophenoxyacetic acid are made on the leaves, roots may appear on the petioles and midribs. When moist conditions exist in the greenhouse, as when the potted plants are closely packed together, the emerging root primordia continue to elongate, giving the plants a distinctly hairy appearance. At the base of the hypocotyl, the roots become fasciated, extending outward as wedges of tissue at right angles to the stem axis.

Following such a heavy application of 2,4-dichlorophenoxyacetic acid, the stoppage of growth is complete except as it involves an internal cell proliferation and differentiation. The young trifoliate leaves do not expand, and they are usually incorporated into the terminal gall, with only the tips of the young leaves extending beyond the end of the gall.

Histological responses

In order to examine adequately the histological effects produced by 2,4-dichlorophenoxyacetic acid on the various tissues of the kidney-bean stem and to determine the responsiveness of tissues of varying ages and maturity, three portions of the stem were selected for study, namely, those portions of the hypocotyl, first internode, and second internode about 5–10 mm. below the nodes. DOUTT (3) had previously determined that the hypocotylar organization immediately below the cotyledons was essentially that of subsequent internodes. The petiole of the primary leaf was briefly studied.

SECOND INTERNODE

The second internode, being relatively immature, responded to a greater degree than did the older tissues of the first internode and the still older tissues of the hypocotyl. The responses will be described in some detail.

At the time of treatment the second internode consisted largely of immature cells (fig. 1). The pith was solid. The protoxylem, radially arranged, was not heavily lignified, and the protoplasts and nuclei were still visible in the outer vessels. Other primary tissues were readily identifiable; but, while a fascicular cambium was evident in some sections, no secondary tissue had been laid down. The ray cells, extending through the primary phloem, showed no evidence of any radial elongation. The pericyclic fibers, two to five cells deep over the vascular bundles, were well differentiated, but their walls were still thin, and their contents had not begun to disintegrate.

In longitudinal section the cortical and
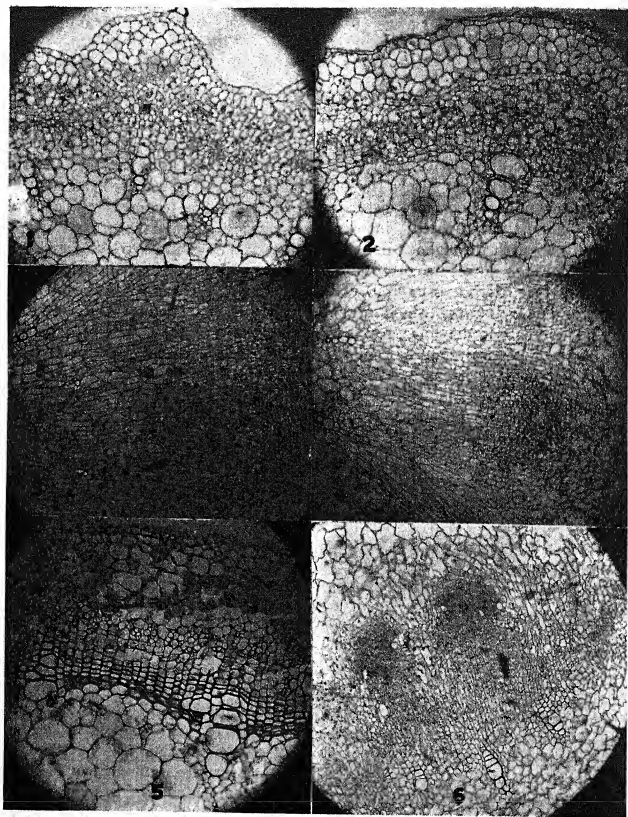
FIGS. 1–6.—Fig. 1, transverse section of the second internode of an untreated kidney bean. Fig. 2, transverse section of second internode of kidney bean, 36 hours after treatment. Interfascicular endodermis, cambium, and ray cells show activity. Fig. 3, transverse section of second internode, 72 hours after treatment. Only primary xylem remains unaffected; other tissues probably embryonic at time of treatment. Fig. 4, second internode, 96 hours after treatment. Root primordia well developed at this stage. Fig. 5, transverse section of first internode of untreated kidney bean. Fig. 6, first internode, 90 hours after treatment. Relationship of root primordia to ray areas clearly shown. Note radial elongation of endodermis and the formation of short, pitted tracheids.

cambial cells were considerably elongated; and the endodermal, parenchymatous, and ray cells were roughly twice as long as they were wide. No marked activity was noticed in any of the tissues, although the meristematic appearance of phloem parenchyma and ray cells indicated that they were still capable of further division.

The first indication of histological activity was evident 9 hours after the application of 2,4-dichlorophenoxyacetic acid. The cytoplasm and nuclei took on a much deeper stain, and the nuclei were enlarged considerably in the endodermis, phloem parenchyma, cambium, xylem parenchyma, and ray cells. A somewhat less marked increase in staining intensity characterized the cells of the pith and the cortex. No perceptible increase in cell size was noted in any of the tissues. No measurements of the longitudinal axes of cells were made to determine which tissue was responsible for the distinct curvature of the stem. Division figures up to metaphase were observed in the phloem parenchyma and ray cells, but the fact that no further division figures were found at 24 hours suggests that these tissues were still sufficiently meristematic to be dividing normally. It appears likely that the application of 2,4-dichlorophenoxyacetic acid hastened the division cycle of those cells which were already undergoing mitosis but that it did not stimulate others to divide until later.

At the end of 24 hours the endodermis had visibly enlarged in a radial direction, and the endodermal nuclei were undergoing enlargement prior to entering early prophase.

Between 36 and 48 hours the endodermis had divided both transversely and tangentially, with the greater number of divisions in the transverse plane (fig. 2). The endodermal cells over the vascular bundles were considerably slower to react than those over the interfascicular regions, dividing some 12–18 hours after the others. The fascicular cambium was equally active at this time; tangential division occurred rapidly in the vicinity of the outer primary vessels. The ray cells and the phloem parenchyma were somewhat slower to respond, but mitotic activity had been initiated. Cortical cells adjacent to the endodermis were beginning to enter prophase, but no activity other than an intense nuclear staining was to be found in the pith and xylem parenchyma.

By 72 hours a considerable mass of proliferated tissue had been produced, particularly by the developing cambium. This tissue, together with the phloem parenchyma, gave rise to a broad zone of rapidly dividing cells, which, as they increased in number and elongated in a radial direction, pushed the primary tissues farther and farther apart (fig. 3). This illustration is from a somewhat younger section of the second internode than those shown in figures 1 and 2, but it is more representative of the reaction of this portion of the stem to 2,4-dichlorophenoxyacetic acid. The entire region between the xylem and the cortex became a mass of proliferating cells, and the identity of the individual tissues, with the exception of the rays, was lost. The ray derivatives may be readily identified by their considerable radial length.

In figure 3 it will be noticed that the pericyclic fibers and the sieve tubes are not distinguishable. It is probable that they were sufficiently immature to respond to the application of 2,4-dichlorophenoxyacetic acid and thus to merge with other proliferating tissues; but in other sections of the second internode, where some differentiation had taken place, these cells did not divide.

No typical phloem or xylem cells were laid down during this period, although short, tracheid-like cells with simple pits were scattered haphazardly throughout the proliferated zone. This zone, at 72 hours, may be thirty to forty cells in depth, as contrasted to the five- to seven-cell thickness of the phloem-cambium region of a normal mature stem. A stem of similar age would still possess a relatively immature secondary xylem and phloem, but it would be clearly recognizable. The 2,4-dichlorophenoxyacetic acid, then, not only stimulates the meristematic tissues into a very rapid division period but, by maintaining meristematic activity in all the cell derivatives, also prevents the maturation of any of the tissues which are normally laid down.

When a stem with the primary tissues fully developed was treated with 2,4-dichlorophenoxyacetic acid, the results were somewht different (cf. figs. 3 and 4). In figure 4, which is a cross-section of an older section of the second internode, the small groups of sieve tubes may be seen over the vascular bundles, with the pericyclic fibers pushed aside by the proliferating ray tissue. The root primordia, which will be discussed in more detail below, arose in the interfascicular regions from the cells of the rays and pushed outward, disrupting the cortex and the epidermis. These root primordia were initiated almost as soon as the ray cells underwent proliferation, but it was not until 72–84 hours after treatment that they could be identified as such with certainty.

Later stages, up to 120 hours, were studied; but, although cell division continued and the root primordia emerged through the cortex, the details added nothing to those already described for earlier stages.

FIRST INTERNODE

The histological study of the first internode was confined to that region just below the primary leaves where the leaf traces had not yet begun to pass outward. This was the portion of the internode which responded initially to 2,4-dichlorophenoxyacetic acid by bending sharply. Figure 5 shows a section of a first internode with the secondary xylem well developed, a condition somewhat more advanced in differentiation than was usual at this stage of development. In most instances the secondary xylem was but one or two cells in thickness.

The pattern of change in the first internode was similar to that in the less mature second internode. The changes, however, were less dramatic. At 24 hours a deeper staining of the endodermis, ray, cambium, and phloem parenchyma was noted. At 48 hours the ray cells and interfascicular endodermis had divided, while the cambium, phloem parenchyma, and fascicular endodermis were in various prophase stages of division. Frequently an entire ray area, from pith to cortex, showed activity. Binucleate cells were often found, indicating that nuclear division progressed at a more rapid rate than did cell division. The 2,4-dichlorophenoxyacetic acid, however, caused no upset in the spindle mechanism or in chromosome-spindle co-ordination. All division figures were monotonously regular.

At 72 hours the cambium and its derivatives, which remain meristematic, had formed a zone of proliferating cells, with a greater degree of proliferation over the interfascicular regions. At 90 hours root primordia were clearly evident (fig. 6), although these had had their initiation somewhat earlier. As nearly as could be determined, it was the

ray cells immediately under the cortex which initiated the formation of root primordia. As the root primordia developed, the core appeared to be derived from the ray cells, as was the region of the histogen, while the flanking regions were made up of derivatives from the cambium and phloem parenchyma. The apex of the root, composed of many small cells, continued to proliferate at a rapid rate, passing through the cortex and epidermis to emerge as a small protuberance on the surface of a swollen stem. The core of the root was generally composed of somewhat elongated cells, with simple-pitted tracheids forming in a scattered fashion along the boundary between the core and the flanking zone. It could not be determined whether the tracheids arose from cells of the ray, the cambium, or the phloem or from all three sources.

Endodermal cells capping the root primordia were disrupted as the root passed outward, and the groups of sieve tubes and pericyclic fibers were pushed to one side. The endodermal cells, however, had undergone a considerable radial elongation before disruption (fig. 6). This was particularly true of those cells which lie over the fascicular regions and which flank the root primordia.

Some differentiation of xylary elements occurred following treatment with 2,4-dichlorophenoxyacetic acid. The cells laid down were not the usual elongated tracheal cells characteristic of the kidney bean but were short, with simple pits, and not very heavily lignified. Similar cells were differentiated in the proliferated zone derived from the cambium (fig. 7). They may form islands of tracheids or may exist singly, and they were more generally formed in the areas between root primordia than at the bases of such roots.

The pith exhibited no response. The xylem parenchyma was only slightly responsive, but occasionally areas were found in which the primary xylem elements were forced apart by the proliferating xylem parenchyma (fig. 8).

Figures 9 and 10 reveal the longitudinal aspects of change which take place in the first internode. In the untreated section (fig. 9) the considerable length of all tissues, with the exception of the endodermis, cortex, and epidermis, is evident. The cambium has not yet laid down any secondary tissues, although it is itself well differentiated. Figure 10, taken in the region of a root primordium, reveals quite clearly some of the changes brought about in response to 2,4-dichlorophenoxyacetic acid. The cambium no longer consists of fusiform elements, since many transverse divisions reduced the length of the cells. Its derivatives matured as short tracheids. The sieve tubes and the pericyclic fibers were unchanged, but the phloem parenchyma and the ray cells divided many times to form the root primordium, which, as it passed outward, disrupted the endodermis, cortex, and epidermis.

HYPOCOTYL

The hypocotyl responded to 2,4-dichlorophenoxyacetic acid in much the same manner as the first internode, except that the tempo of change was slower.

Figure 11 is a cross-section of an untreated stem. Figure 12 shows the changes which took place 72 hours after treatment. The endodermis appeared to divide more frequently in the hypocotyl than in other sections of the stem, but the other changes were similar, except that the pattern of root formation was somewhat different. In the first internode there were fifteen to twenty root primordia, arranged in a spokelike fashion
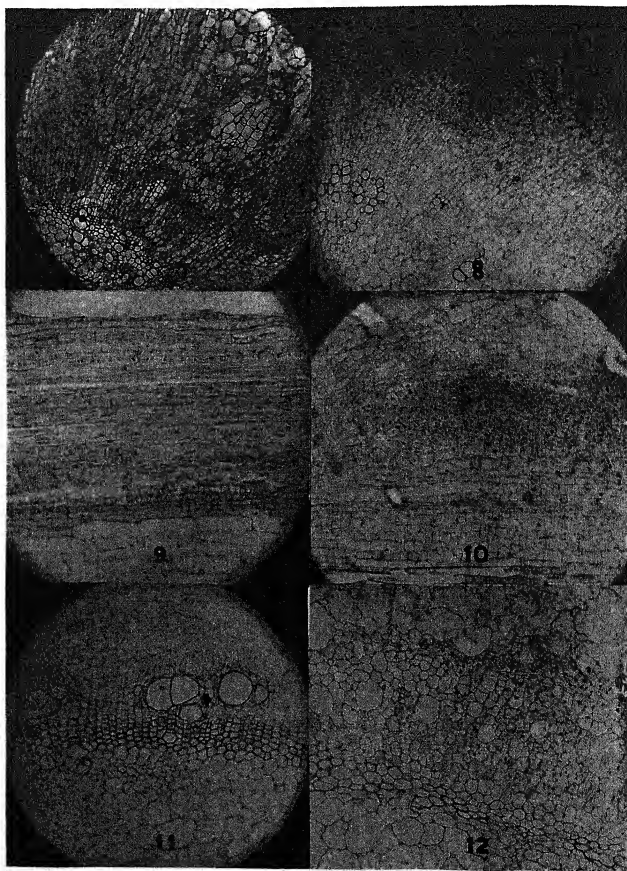
FIGS. 7–12.—Fig. 7, first internode, 120 hours after treatment. Note groups of xylary elements differentiated in the proliferated zone. Fig. 8, first internode, 96 hours after treatment. Proliferation of xylem parenchyma has disorganized the arrangement of elements in the vascular bundle. Fig. 9, longitudinal section of first internode of an untreated bean plant. Fig. 10, longitudinal section of first internode, 84 hours after treatment. FIG. 11, transverse section of the hypocotyl of an untreated kidney bean. Fig. 12, transverse section of hypocotyl, 72 hours after treatment.

(fig. 13). In the hypocotyl a distinct tetrarch arrangement was usually found (fig. 14). This is undoubtedly a reflection of the tetrarch system, which characterizes the bean root. Between the root primordia there was a much greater produc-

PETIOLE OF THE PRIMARY LEAF

The sequence of histological events of this portion of the plant has not been investigated so thoroughly as have the stem portions previously described, but a comparison of figures 16 and 17 will re-
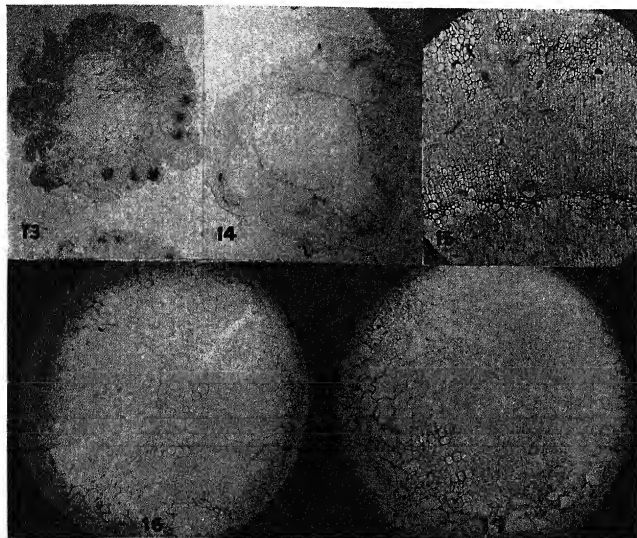
FIGS. 13–17.—Fig. 13, first internode, 120 hours after treatment, showing the numerous root primordia which form between the vascular bundles. Fig. 14, hypocotyl, 120 hours after treatment, showing the tetrarch arrangement of the root primordia (one group of primordia has been lost). Fig. 15, hypocotyl, 120 hours after treatment, showing the differentiation of tracheid-like elements in the proliferated zone. Fig. 16, transverse section of an untreated petiole. Fig. 17, petiole, 96 hours after treatment, showing proliferation occurring in the phloem-cambium region.

tion of tracheal elements in the proliferated zone than was found in the younger portions of the stem, and it was considerably more in evidence at the base of the hypocotyl than at the top (fig. 15).

veal the changes that have taken place. Figure 17 shows that the phloem-cambium region had undergone a considerable enlargement due to the rapid proliferation of cells. This expansion displaced the pericyclic fibers, and the di-

viding cells invaded the fundamental parenchyma surrounding the bundles. There had been some stimulation of the fundamental parenchyma over and between the bundles, but, in general, this tissue did not show any marked responsiveness. The xylem parenchyma was slightly responsive. No xylary elements appeared to be differentiated in the petiole following the treatment.

Discussion

It has been adequately shown (1, 2, 4, 8, 10, 11, 12, 13) that 2,4-dichlorophenoxyacetic acid and other substituted phenoxyacetic acids differ from other growth-regulating substances in their influence on formative disturbances when used in extreme dilutions and in the systemic nature of their effects. The combined effect of these influences on the physiological balance within the plant undoubtedly determines the unique properties which characterize 2,4-dichlorophenoxyacetic acid as a herbicidal agent. In other respects the physiological action of this compound bears a close similarity to that of the more localized growth-regulating substances, in that the formative disturbances are somewhat of the same nature as regards tissue responses and cell proliferation. However, as MULLISON (9) indicates in a table comparing the histological responses of growth-regulating substances tested on the kidney bean, each of the substances tested to date produces a sufficiently characteristic pattern of events as to suggest that each sets in motion, interrupts, or alters a pattern of physiological mechanisms peculiar to itself. The 2,4-dichlorophenoxyacetic acid, in turn, shares this individuality of effect, although as yet no data from this study, or from other investigations, yield any clue as to the mechanisms involved.

The 2,4-dichlorophenoxyacetic acid produces histological responses very similar to those induced by indoleacetic acid (6) and l-tryptophane (5). The endodermal, phloem, ray, and cambial cells are greatly activated by all three; but 2,4-dichlorophenoxyacetic acid does not stimulate the formation of vascular bundles in the endodermis and phloem, being similar in this respect to tetrahydrofurfural butyrate (9). Alpha-naphthalene acetamide, on the other hand, has very little effect on the phloem (7), while 2,4-dichlorophenoxyacetic acid is equally ineffective in stimulating the xylem, xylem parenchyma, and pith. Like indoleacetic acid and alpha-naphthalene acetamide but unlike tetrahydrofurfural butyrate and l-tryptophane, 2,4-dichlorophenoxyacetic acid induces considerable root formation. It is evident, therefore, that, while similarities of response exist, differences of response are equally obvious.

From the point of view of herbicidal effectiveness, 2,4-dichlorophenoxyacetic acid and others of a somewhat similar chemical structure rank high among the so-called "weed-killers" because of their severe inhibitory action on broadleaf plants at extreme dilutions and at low rates of application (2, 4, 10, 11, 12, 13). Unpublished observations on a number of the substituted phenoxyacetic acids and their derivatives tend to suggest that the greater the degree of cell proliferation and formative disturbance induced by a substance, the more effective is that substance as a herbicidal agent. Histological studies, therefore, provide an excellent criterion of effect when comparative observations are being made on the herbicidal qualities of various growth-regulating substances; but they must be supplemented by other studies, since the selective herbicidal action of these substances on various plant species is a recognized fact (2, 10, 11).

Summary

1. Aqueous-spray applications of a 0.1% solution of 2,4-dichlorophenoxy-acetic acid induce a considerable degree of cell proliferation in the stem of the kidney-bean plant.

2. The histological changes induced are as follows:

> *Epidermis.*—No response.
>
> *Cortex.*—Little response, although cells adjacent to the endodermis frequently enlarge and divide.
>
> *Endodermis.*—Active; transverse and tangential divisions occur, as well as considerable radial elongation.
>
> *Pericyclic fibers.*—Active if embryonic, but show no response if differentiated.
>
> *Phloem.*—Sieve tubes inactive; phloem parenchyma very active and may enter into root formation or differentiate into isolated tracheal groups.
>
> *Cambium.*—Very active; derivatives remain meristematic, no phloem or xylem being regularly differentiated, although tracheal groups may be laid down; takes part in root formation.
>
> *Rays.*—Very active, being the primary tissue involved in root formation.
>
> *Xylem.*—Formation of xylary elements greatly inhibited and, when occurring, much disorganized; xylem parenchyma slightly active, but to no great extent.
>
> *Pith.*—No response.

3. In general, it may be stated that meristematic tissues and those capable of reverting to a meristematic condition are most readily affected by 2,4-dichloro-phenoxyacetic acid. The derivatives of such tissues remain meristematic for considerable periods, and, if differentiation occurs, it is never in an orderly fashion.

4. The effect of 2,4-dichlorophenoxy-acetic acid is systemic in nature, even at relatively low concentrations. In this respect it differs from other growth-regulating substances.

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EFFECT OF SPRAY APPLICATIONS OF 2,4-DICHLOROPHE-NOXYACETIC ACID ON SUBSEQUENT GROWTH OF VARIOUS PARTS OF RED KIDNEY BEAN AND SOYBEAN PLANTS[1]

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Introduction

Small quantities of 2,4-dichlorophenoxyacetic acid produce marked responses in plants when applied in amounts less than lethal. Some of the responses produced in the red kidney bean plant have been described by SWANSON (2). The various plant parts are affected to different degrees. The present investigations were concerned with growth and weight changes produced by this compound in various parts of kidney bean and soybean plants and had as their prime objective the determination of the best and simplest criterion that could be used experimentally as an index of overall growth-inhibitory effect in the evaluation of the herbicidal activity of growth-regulating substances.

Experimentation

EXPERIMENT I: RED KIDNEY BEAN

Red kidney beans were planted in 4-inch pots, and before treatment were thinned to two plants of uniform size per pot. The plants were grown under ordinary greenhouse conditions and were treated 14 days after planting, when the first trifoliate leaves were expanding.

Three aqueous solutions of the growth regulator were prepared, each of which contained 0.5% Carbowax 1500. The plants were sprayed in 50-ml. amounts at rates of 0.001, 0.01, and 0.1 gm. per square yard of plot area with 200-ml.

pressure-sprayers under 100 lb. pressure. Plants treated with the lowest amount (0.001 gm. per sq. yd.) appeared only slightly affected. Curvature of shoots and leaf epinasty occurred soon after treatment. Subsequent growth seemed little retarded, although formation of leaves with abnormal veination occurred. Plants treated with the highest concentration were severely inhibited. Shoot curvature and epinasty were soon manifested, and further growth was much inhibited. Thickening of stems and petioles occurred, and gall-like growths at the nodes often formed. Terminal growth often ceased and the plants appeared to be "frozen," since growth in length ceased. The plants were dead and withered 21 days after treatment. Plants treated with an intermediate amount (0.01 gm. per sq. yd.) manifested intermediate symptoms.

For each treatment four pots of two plants each were used. At time of treatment four pots of plants were harvested and measurements made. Five, 10, 15, 21, and 31 days after treatment, sets of treated and one set of control plants were harvested. The stages of development of control plants at the various times of harvest were as follows:

TIME HARVEST*   STAGE DEVELOPMENT OF CONTROLS
0[†]......First trifoliate leaf expanding
5.......Second trifoliate leaf expanding
10.......Third trifoliate leaf expanding
15.......Flowers opening; four trifoliate leaves
21.......Five trifoliate leaves
31.......Pods formed

  * Days after treatment.
  † Time of treatment.

At all harvests several linear measurements were made, the plant was sectioned, and the various parts weighed. The fresh tissues were then dried in an oven for 3 days at about 70° C. to obtain dry weights. Length measurements were the average of the two plants in each pot, and the weights were the sum of the two plants. With few exceptions, length measurements were made of the following parts: total height of plant (to growing point), length of petiole of second trifoliate leaf, and length and width of middle leaflet of second trifoliate leaf. Weights were taken of hypocotyl, first internode, primary leaf blades, petioles of primary leaves, stem above second node (without leaves), trifoliate leaf blades, petioles of trifoliate leaves, roots (plus hypocotyl below soil), and pods. By adding the weights of the appropriate parts, the total weights of various plant portions were obtained.

1. HYPOCOTYL AND FIRST INTERNODE. —Treatment with the growth regulator caused these plant parts to increase greatly in diameter. Their increase in fresh weights was due largely to increase of water content of the tissue (figs. 1, 2). Figures 1 and 2 indicate that the first internodes of treated plants did increase in dry weight but that hypocotyls showed no such increase. It has been shown that stem proliferation is largely due to formation of meristematic tissues, which have little dry weight (2). Decrease in dry weight of hypocotyls of treated plants may be due to cell degeneration.

2. PRIMARY LEAF BLADES.—Primary leaves of treated plants remained green and were still attached to the plant long after primary leaves of control plants had withered and fallen (fig. 3). Exceptions were leaves of plants receiving the highest level (0.1 gm. per sq. yd.) of the growth substance, as these plants were

dead and withered 21 days after treatment. Figure 3 indicates that primary leaves of control plants have mostly withered and defoliated 21 days after treatment. The dry weights of primary leaves when plotted showed the same
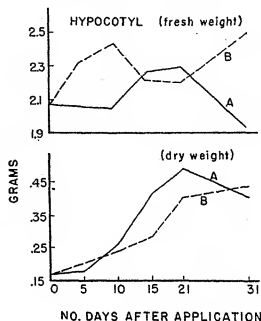
FIG. 1.—Average fresh and dry weights on a two-plant basis of hypocotyls of control plants (A) and of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.01 gm. per square yard (B).
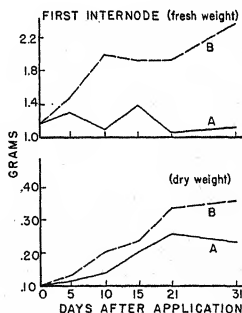
FIG. 2.—Average fresh and dry weights on a two-plant basis of first internodes of control plants (A) and of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.01 gm. per square yard (B).

trend as fresh weights, and hence are not presented.

3. TRIFOLIATE LEAF BLADES, TRIFOLIATE LEAVES, AND WEIGHT OF PLANT ABOVE SECOND NODE.—Treatment was followed by a lowered fresh weight of trifoliate leaf blades, and there was a close correlation between level of growth
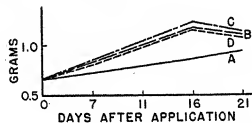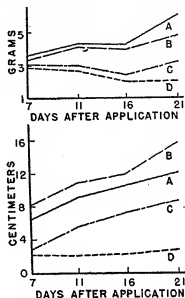

FIGS. 3, 4.—Fig. 3 (above), average fresh weight on a two-plant basis of primary leaf blades of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control. Fig. 4 (below), average fresh weight on a two-plant basis of trifoliate leaf blades of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

regulator applied and decrease in weight of trifoliate leaf blades produced (fig. 4). A final decrease in weight of trifoliate leaf blades of controls and plants treated with the lowest level of the compound was due to loss of lower leaves by withering and defoliation. Statistical analysis showed that differences in fresh weight of trifoliate leaf blades in controls and treated plants, and of plants treated by different levels of the compound, were

highly significant 10 days after treatment. Fresh-weight determinations on trifoliate leaves gave results very similar to those on trifoliate leaf blades. Graphs of weights of trifoliate leaf blades or leaves on a dry-weight basis showed trends very similar to those plotted on a fresh-weight basis.

In a study of the relative inhibitory effects of growth regulators on young red kidney beans, the fresh weight of trifoliate leaf blades or leaves which are produced would appear to be a sensitive criterion. Many growth regulators cause proliferations and swellings on stems which may greatly increase the fresh-plant weight. However, such weight increases may not be indicative of plant recovery. The fresh weight of that part of the plant produced above the second node has been used as a measure of growth inhibition or stimulation in red kidney bean (1), and the results of the present experiment confirm its value for this purpose. Ten days after treatment there were significant differences between controls and treated plants, and between treated plants, when the fresh weight of plants above the second node was used as a criterion. The plotted data have the same general trend as those of figure 4.

Measurements of the length and width of the middle leaflet of the second trifoliate leaf, which was the first leaf produced after treatment, indicate that the growth regulator inhibited leaf expansion in proportion to the amount used. Figure 5 shows the progressive growth in length of the leaflet. Growth in width closely paralleled length.

Lengths of petioles of the second trifoliate leaves were also measured. The lowest rate of treatment had little effect on growth in length of the petioles, but the two higher levels greatly decreased it (fig. 6).

4. TOPS.—The fresh weights of tops were decreased by treatment with the growth regulator. Figure 7 indicates that the differences between controls and treatments and between different treatments were often small. This may be because the greatly increased weight of treated plants was due to proliferated hypocotyls, first internodes, and stems.

Heights of plants were measured from the soil to the growing point. In general,

2,4-dichlorophenoxyacetic acid decreased plant heights, but at 10 days after treatment there was little difference between plants treated with 0.001 and with 0.01 gm. per sq. yd. of agent (fig. 8).

5. ROOTS (INCLUDING HYPOCOTYL BELOW SOIL SURFACE).—Figure 9 indicates

FIGS. 7, 8.—Fig. 7 (above), average fresh weight on a two-plant basis of tops of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control. Fig. 8 (below), average height of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

FIGS. 5, 6.—Fig. 5 (above), average length of the middle leaflet of the second trifoliate leaf of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control. Fig. 6 (below), average length of the petiole of the second trifoliate leaf of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

that the compound, at rates of 0.1 and 0.01 gm. per sq. yd., decreased the amount of roots on a dry-weight basis, but that the lowest level (0.001 gm. per sq. yd.) had little effect.

6. SHOOT-TO-ROOT RATIO.—There was little effect on shoot-to-root ratio when sublethal amounts of the compound were used (0.001 or 0.01 gm. per sq. yd.). The ratio gradually increased from about 2.7 at time of treatment until a maximum of about 3.7 was reached 21 days after treatment.

7. Pods.—The growth regulator decreased and delayed pod production (table 1). After 31 days, controls produced about 2.8 times more pods on a fresh-weight basis than did plants treated with 0.001 gm. per sq. yd. of the compound. However, when pod development
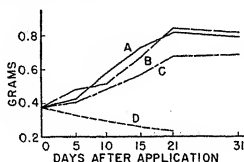
Fig. 9.—Average dry weight on a two-plant basis of roots (plus hypocotyl below soil surface) of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

TABLE 1

FRESH WEIGHT (GM.) OF PODS OF RED KIDNEY BEANS AT THREE STAGES OF DEVELOPMENT

TREATMENT (GM./SQ. YD.)	DATE OF HARVEST (DAYS AFTER TREATMENT)		
	21	31	40
Control	0.24	7.29	8.54
0.001	0.02	2.56	6.30
0.01	0	0.2	2.40
0.1	0	0	0

was allowed to continue 9 more days, controls produced only about 1.4 times the weight of pods as treated plants.

EXPERIMENT II: SOYBEAN

Illini soybeans were planted in 4- and 6-inch pots and before treatment were thinned to three plants per pot. Plants were treated on October 17, 1944 (20 days after planting), when the second trifoliate leaves were expanding.

The method of treatment was similar to that of experiment I, except that no Carbowax was used in the solutions. The growth of plants treated with 0.1 gm. per sq. yd. was severely inhibited, while plants treated with 0.001 gm. seemed only slightly affected. Plants treated with 0.01 gm. per sq. yd. were moderately inhibited. Treated soybeans manifested symptoms similar to those of treated kidney beans.

At the time of treatment, and at 7, 11, 16, and 21 days after treatment, plants in 4-inch pots were harvested. Thirty-four and 41 days after treatment, plants in 6-inch pots were harvested. The stage of growth of control plants at the various harvests were:

TIME OF HARVEST*	STAGE OF DEVELOPMENT OF CONTROLS
0†	Second trifoliate leaf expanding
7	Third trifoliate leaf expanding
11	Fourth trifoliate leaf expanding
16	Flowers forming
21	Flowering; five trifoliate leaves
34	Fruiting initiating
41	Many pods formed

* Days after treatment.
† Time of treatment.

At all harvests the same length and weight measurements were taken as in experiment I. All weight figures are means of four replicate pots on a three-plant basis, and length measurements are the means of twelve plants.

1. HYPOCOTYL AND FIRST INTERNODE.—The 2,4-dichlorophenoxyacetic acid caused these parts to increase greatly in diameter. The resultant fresh-weight increase was due, as in case of kidney beans, largely to increased water content of the tissues (fig. 10). For example, 11 days after treatment hypocotyls of control plants and plants sprayed by the compound at 0.01 gm. per sq. yd. each gave dry weights of 0.17 gm., but their fresh weights were 0.84 and 1.21 gm., respectively.

2. PRIMARY LEAVES.—Primary leaves of treated plants became heavier, but those of controls were little changed (fig. 11). The results are similar to those obtained with kidney bean. Graphs based on dry weights were similar to figure 11.

3. TRIFOLIATE LEAF BLADES, TRIFOLIATE LEAVES, AND WEIGHT OF PLANT
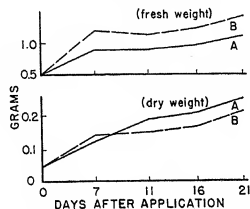
FIG. 10.—Average fresh and dry weight of hypocotyls of controls and soybean plants sprayed with 2,4-dichlorophenoxyacetic acid at the rate of 0.1 gm. per square yard, B. Control, A. Figures are on a three-plant basis.

FIG. 11.—Average fresh weight on a three-plant basis of primary leaves of soybean after application of 2,4-dichlorophenoxyacetic acid, at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

ABOVE SECOND NODE.—The total fresh weight of leaf blades of treated plants was less than that of control leaf blades, and there was close correlation between decrease in weight and increase in amount of compound used (fig. 12). When fresh weights of trifoliate leaves were plotted, the same general relation existed between fresh weights and amount of compound used; but at certain harvests there were no differences in

controls and plants treated with 0.001 gm. per sq. yd., probably because the petioles of treated plants swelled and increased the total leaf weights. Fresh weights of plants above the second node also gave good correlation between decreased weights and increasing amounts of compound. However, differences in weights were often small. In general,

FIGS. 12, 13.—Fig. 12 (above), average fresh weight on a three-plant basis of trifoliate leaf blades of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control. Fig. 13 (below), average length of the petiole of the second trifoliate leaf of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

data based on a dry-weight basis showed trends similar to those shown in figure 12.

This experiment indicates that fresh weight of trifoliate leaf blades would be the best criterion in a study of inhibitory effects of a growth regulator on soybeans. Twenty-one days after treatment there were significant differences between controls and treated plants and between plants that received various amounts of compound, when fresh weight of leaf blades was used as a criterion. This was

not true for any other of the criteria studied.

The lengths of the middle leaflets of the second and third trifoliate leaves were less in treated plants, and the higher the treatment rate used, the shorter were the leaflets. However, the length of the petiole of the second trifoliate leaf was increased by the lowest level of the compound, although the two higher levels inhibited growth in length (fig. 13).
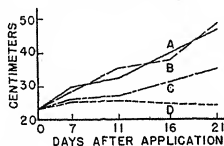
FIG. 14.—Average height of soybean, as measured from soil to the growing point, after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.
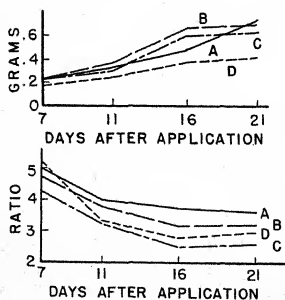
FIGS. 15, 16.—Fig. 15 (above), average dry weight on a three-plant basis of roots (plus hypocotyl below soil surface) of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control. Fig. 16 (below), average ratio (dry-weight basis) of shoot to root of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (B), 0.01 gm. (C), and 0.1 gm. (D) per square yard. A, control.

4. TOPS.—The total weight of tops was decreased by application of the growth regulator. However, for 16 days after treatment there was little difference in weight of controls and of plants treated at 0.001 gm. per sq. yd. Plant height was little affected by the smallest amounts of the compound, but length was inhibited by higher rates of application (fig. 14).

5. ROOTS (INCLUDING HYPOCOTYL BELOW SOIL SURFACE).—Dry weights of roots of plants treated with the two smaller amounts of the compound significantly increased when measured 16 days after

TABLE 2

FRESH WEIGHT (GM.) OF PODS OF SOYBEANS AT TWO STAGES OF DEVELOPMENT

TREATMENT (GM./SQ. YD.)	DATE OF HARVEST (DAYS AFTER TREATMENT)	
	34	41
Control	3.70	7.70
0.001	1.76	6.41
0.01	0.56	3.26
0.1	0.03	0.52

treatment (fig. 15). After 21 days dry weights of roots of control plants had increased and were about the same as those of plants that received the two lowest treatment levels.

6. SHOOT-TO-ROOT RATIO.—On a dry-weight basis this was decreased by all treatments (fig. 16). Plants treated with the largest amount had a greater such ratio initially than had the control plants, but this soon fell below the control.

7. PODS.—Production was decreased and delayed by the growth regulator (table 2). The delay in pod formation is illustrated by the fact that 34 days after treatment the controls produced 2.1 times the weight of pods of plants treated

with 0.001 gm. of compound per sq. yd. but that this ratio was only 1.2 at the harvest 41 days after treatment.

Summary

1. Plots containing young red kidney bean and soybean plants were sprayed with 2,4-dichlorophenoxyacetic acid at rates of 0.1, 0.01, and 0.001 gm. per sq. yd. The effects of the agent on the growth of various plant parts were studied. Linear, and fresh and dry weight measurements were obtained at several intervals following treatment.

2. Hypocotyls and first internodes increased greatly in diameter. The increase in fresh weight was due mostly to increased water content.

3. Primary leaves of treated plants were much heavier than those of controls. Those of red kidney beans remained green and attached to the plants long after those of controls had withered and fallen.

4. Total weights of trifoliate leaf blades, trifoliate leaves, and weight of plants above second node were decreased by the growth regulator. There was good correlation between decrease in weight and increase in amount of compound applied. These criteria would be excellent in studying the relative inhibitory or stimulating actions of growth regulators on red kidney beans. Ten days after treatment there were highly significant differences between controls and treated plants, and between treated plants, when fresh weights of trifoliate leaves or leaf blades were used as criteria. The differences were significant when the fresh weight of kidney bean plants above the second node was used as a criterion. With soybeans, only fresh weight of trifoliate leaf blades gave significant differences between controls and treated plants and between plants that received various rates of compound when plants were harvested 21 days after treatment.

5. The growth in length and width of certain leaflets which developed after treatment was inhibited by the growth regulator. The two higher levels of compound (0.1 or 0.01 gm. per sq. yd.) inhibited the linear growth of certain petioles. The lowest level (0.001 gm. per sq. yd.) stimulated growth in length of a soybean petiole but had little effect on a bean petiole.

6 The fresh weight of tops was decreased by the growth regulator. Heights were usually decreased but differences caused by various treatments were sometimes small.

7. The compound at rates of 0.01 and 0.1 gm. per sq. yd. decreased dry weight of roots of kidney bean, but 0.001 gm. had little effect. With soybeans the two lower rates of application significantly increased weight of roots at 16 days after treatment. However, by 21 days the weight of control roots was about the same as that of the treated plants.

8. With kidney beans there was little effect on the shoot-to-root ratio when two lower amounts of the compound were used. With soybeans, the ratio was reduced by all treatments.

9. The growth regulator delayed the onset and decreased the amount of pod formation.

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INFLUENCE OF RAINFALL ON THE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID SPRAYED FOR HERBICIDAL PURPOSES[1]

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Introduction

Since it has been shown that several hours are required for plants to absorb maximum amounts of 2,4-dichlorophenoxyacetic acid sprayed on their leaves (3), an investigation was undertaken to determine to what extent heavy rainfall would remove this compound and reduce its effectiveness as a herbicide. These studies were conducted by means of greenhouse and field experiments in which either aqueous or oil solutions of the compound were sprayed on the plants, which were thereafter exposed at various intervals to heavy artificial rainfall.

Experimentation

GREENHOUSE TRIALS

Three experiments were conducted in the greenhouse, one with soybean and two with red kidney bean. All plants were grown in 4-inch pots. Before treatment with 2,4-dichlorophenoxyacetic acid or its ammonium salt, the soybeans were thinned to three and red kidney beans to two per pot; at time of treatment the soybeans had expanded two trifoliate leaves and the red kidney beans, one. Three replicate pots were used for each test. The herbicide was applied as a spray, using 200 ml. air-pressure pumps or a De Vilbiss atomizer. Artificial rainfall of 1 inch was applied in about 5 minutes with a garden-hose noz-

zle. The water spray was directed upward so that drops of medium size fell almost vertically.

EXPERIMENT I.—Young soybean plants were sprayed with an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid, or with a solution of the acid in oil, in the amount of 0.1 gm. per square yard of plot surface, in 50 ml. of solution. Spraying was done with 200-ml. pressure pumps. The oil emulsion was prepared by dissolving 1 gm. of the herbicide in 50 ml. of diethylene oxide (Dioxan) and adding 450 ml. of no. 2 diesel fuel oil. Plants were subjected to artificial rainfall at intervals of $\frac{1}{4}$, 1, 3, 6, 12, 24, and 48 hours after treatment. Some treated plants received no artificial rainfall.

All plants sprayed by the herbicide in oil were dead and withered several days after treatment, whether subjected to artificial rainfall or not. Plants sprayed with the aqueous solution were harvested 3 weeks after treatment, when the controls had four or five trifoliate leaves and were fruiting. The fresh weight of trifoliate leaf blades was obtained, as it has been shown that this is an excellent criterion to measure differences in growth inhibition caused by growth regulators of this type (2). The average fresh weights of trifoliate leaf blades on a three-plant basis for control plants and for plants subjected to artificial rainfall $\frac{1}{4}$, 1, 3, 6, 12, 24, and 48 hours after treatment and for treated plants not subjected to rainfall were 4.93, 4.14, 3.84, 3.14, 2.01, 1.66,
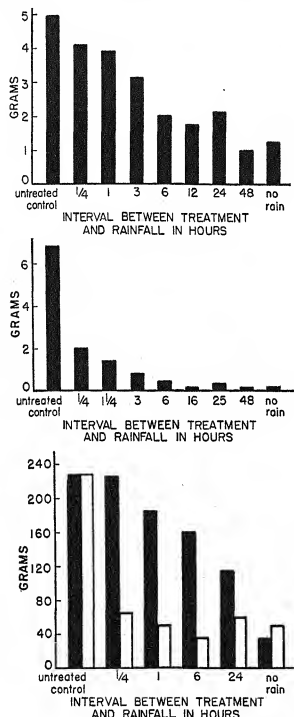
FIGS. 1, 2, 3.—Fig. 1 (upper), average fresh weight on a 3-plant basis of trifoliate leaf blades of soybean sprayed by the ammonium salt of 2,4-dichlorophenoxyacetic acid in water at rate of 0.1 gm. per square yard and subjected to rainfall at various intervals after treatment. Fig. 2 (middle), average fresh weights on a 2-plant basis of trifoliate leaf blades of red kidney bean sprayed with the ammonium salt of 2,4-dichlorophenoxyacetic acid in water at rate of 0.1 gm. per square yard and subjected to rainfall at various intervals after treatment. Each treatment is the average of three replicate pots. Fig. 3 (lower), influence of rainfall on effectiveness of ammonium 2,4-dichlorophenoxyacetate in aqueous (black) and of the acid in oil (white) solution upon yield of soybeans. Figures are mean yields per plot (28 row feet) on air-dry basis.

2.17, 0.97, and 1.24 gm., respectively (fig. 1). Only control plants were fruiting, and, in general, the longer the time interval between treatment and rainfall, the less was the recovery made. Figure 1 indicates that plants subjected to rain $\frac{1}{4}$ hour after treatment were markedly stunted, and that recovery was slight when 6 hours or more had elapsed before treated plants were subjected to rain.

EXPERIMENT II.—Young red kidney bean plants were sprayed with the ammonium salt of 2,4-dichlorophenoxyacetic acid in aqueous solution or with the acid form of this compound dissolved in oil at a rate of 0.1 gm. per square yard, in 10 ml. of liquid. The oil solution was prepared by dissolving 1 gm. of the herbicide in 2 ml. of tributylphosphate and then bringing the solution to 100 ml. volume with no. 2 diesel fuel oil (1). One-fourth, $1\frac{1}{4}$, 3, 6, 16, 25, and 48 hours after treatment, plants were subjected to artificial rainfall. The plants were harvested 12 days after treatment, when the controls had four trifoliate leaves. The results were similar to those of experiment I. All plants sprayed with the oil solution died several days after treatment, irrespective of the interval elapsing before being subjected to artificial rain. The average fresh weights of trifoliate leaf blades on a two-plant basis for controls and for plants receiving rain $\frac{1}{4}$, $1\frac{1}{4}$, 3, 6, 16, 25, and 48 hours after being sprayed by the aqueous herbicidal solution, and for treated plants receiving no rain, were 7.06, 1.86, 1.40, 0.96, 0.44, 0.11, 0.25, 0.10, and 0.14 gm., respectively (fig. 2).

EXPERIMENT III.—This experiment with red kidney beans was similar to experiment II, except that a lower rate of application of herbicide (0.01 gm. per square yard) was used. In general, results were in agreement with those obtained in previous experiments. Unless

rainfall followed almost immediately after application of the aqueous spray, little recovery was made (fig. 4). Oil-treated plants made no recovery.

FIELD TRIAL

On May 1, 1945, Chief soybeans were drilled in rows 18 inches apart. At time of treatment (July 13), plants were in the early flowering stage and 24–30 inches in height. They were sprayed with an aqueous solution of the ammonium salt

of 2,4-dichlorophenoxyacetic acid or an oil solution of the acid form at the rate of 0.1 gm. in 10 ml. of solution per square yard of plot. Spray was applied with a De Vilbiss spray gun. The no. 2 diesel-oil solution contained 2% tributylphosphate. The sprays were applied at about 9:30 A.M. and the weather was clear and sunny.

At intervals of $\frac{1}{4}$, 1, 6, and 24 hours after treatment, plants were subjected to a $\frac{1}{2}$-inch artificial rainfall delivered in a
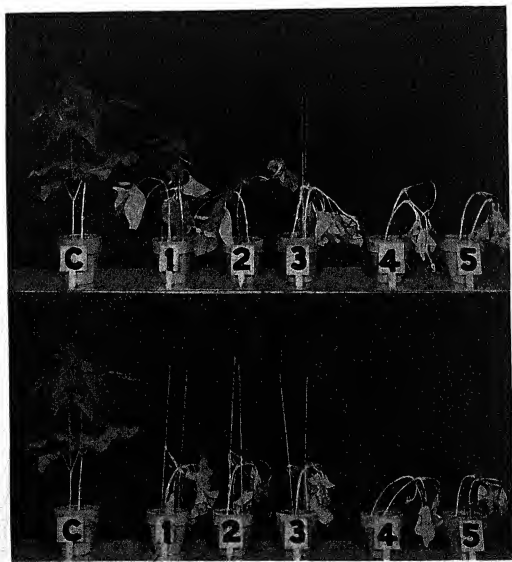
FIG. 4.—Red kidney beans 10 days after treatment with an aqueous solution of ammonium salt of 2,4-dichlorophenoxyacetic acid (*upper*) and with the acid form of this compound in oil (*lower*). C, control. Plants in pots 1, 2, 3, and 4 received artificial rain $\frac{1}{4}$, 1, 3, or 12 hours after treatment. Plants in pot 5 received no rain. Some plants treated with aqueous solution made partial recovery, but all plants treated with oil solution were almost dead.

period of about 13 minutes. Additional plants treated with herbicide received no such rainfall. Four-gallon pump-type fire extinguishers were used to produce the rain. A driving spray was secured when the jet of water struck the baffle attached near the nozzle. A heavy rain was thus produced which was probably more severe than most that occur naturally. Eight hours after the 24-hour period had elapsed a natural rain began, and in a period of about 7 hours 3.3 inches fell.

Plots consisted of four rows 7 feet long and 18 inches apart. All treatments were in quadruplicate, and plots were randomized so that the results could be treated statistically.

Soybeans were harvested September 27, 1945, 76 days after treatment. The pods were threshed and air-dry weights of seeds obtained. The data in table 1 indicate that when this herbicide is applied in oil, an immediate violent rainstorm would cause little if any diminution in its effectiveness (fig. 3). However, when aqueous sprays are applied, early rainfall decreases the effectiveness of the herbicide, and the longer the interval between treatment and rain, the more effective is the herbicide. This experiment indicates that any rain falling within 24 hours of treatment is likely to reduce to some extent the effectiveness of aqueous sprays.

Discussion and Summary

1. The influence of artificial rainfall on the effectiveness of 2,4-dichlorophenoxyacetic acid in oil or an aqueous solution of its ammonium salt sprayed for herbicidal purposes was studied. In greenhouse experiments, young soybean and red kidney bean plants were used, and in the field soybean plants at early flowering stage.

2. When the growth regulator was applied in oil solution, an immediate heavy rain caused no diminution in the response to the herbicide, as drops of rain were apparently shed from the oil-covered leaves without removal of the oil solution. When aqueous solutions of the compound were used, rainfall often decreased plant response (inhibition). In greenhouse experiments, the plant responses were not decreased by an artificial rainfall if 6 hours or more elapsed between application of the compound and artificial rain, but in

TABLE 1

EFFECT OF RAINFALL ON SOYBEANS SPRAYED WITH AQUEOUS SOLUTION OF AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID OR THE ACID IN OIL SOLUTIONS (FIGURES ARE MEAN YIELDS OF BEANS IN GRAMS PER PLOT OF 28-ROW FEET)*

CARRIER	UN-TREAT-ED CON-TROL	TREATED PLANTS				
		Interval between treatment and rainfall (hours)				
		½	1	6	24	No rain
Aqueous....	227.8	226.8	191.8	160.3	116.5	35.0
Oil.........	......	63.3	49.8	35.5	61.8	49.5

* Significant difference between means at 5% is 54.33 gm. Highly significant difference between means at 1% is 73.41 gm.

a field trial rainfall occurring within 24 hours of application reduced effectiveness of the herbicide. This difference in effect of rain is attributed to the varying developmental stages of test plants (4) or to dissimilar environmental conditions of greenhouse and field.

3. The use of an oil carrier for a herbicide may be advantageous in regions of frequent heavy rainfall. Since all artificial rains in these experiments were much heavier than natural rainfall, trials using lower intensities of simulated rain would be of value when aqueous sprays of growth regulators are applied.

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QUANTITATIVE ASPECTS OF AQUEOUS-SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID FOR HERBICIDAL PURPOSES[1]

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Introduction

The use of sprays containing growth-regulating compounds for herbicidal purposes is a field of increasing importance, and it is desirable to obtain information

TABLE 1

SETTINGS OF THE DEVILBISS SPRAY GUN AND DROPLET SIZE IN THE AQUEOUS SPRAYS DELIVERED

SETTING NO.	FLUID NEEDLE ADJUSTMENT (TURNS)	PRESSURE (POUNDS PER SQUARE INCH)	DROPLET SIZE	
			Av. diameter (μ)	Coeff. var. (per cent)
1........	0	10	561 ± 373	66.4
2........	0	20	250 ± 138	55.2
3........	6	10	70 ± 38	54.5
4........	8	40	30 ± 14	49.6

on the relation between some of the characteristics of the spray and the plant response. The purpose of these experiments was to investigate certain features, such as volume, concentration, amount of

[1] Studies conducted at Camp Detrick, Frederick, Md., from August, 1944, to February, 1945, under the supervision of Dr. A. G. Norman.

compound, droplet size, and degree of interception, and to study the extent to which they influence the growth-inhibiting quality of the sprays.

Methods

Sprays were applied with a CV-type DeVilbiss paint-spray gun, fitted with a no. 90 air cap and F fluid tip and needle. Four combinations of adjustments and pressure were used to produce different types of sprays (table 1).

The apparatus was calibrated, in order that known volumes of spray of definite droplet characteristics could be delivered per unit of time. A chamber with a basal area of 196 square inches and sufficiently tall to permit making applications from a height of 4 feet was used for all spray treatments.

Results

VOLUME-CONCENTRATION STUDIES

Differential responses of plants to treatments with a constant amount of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in aqueous

sprays of different volume and concentration were studied. The experimental plants used were kidney beans grown in the greenhouse to the stage of development at which the first trifoliate leaf was beginning to unfold. The compound was applied at a rate of 2.5 mg. per square yard, which was sufficient to produce threshold effects. Green weight of leaf blades of new growth, 17 days after treatment, was the criterion used for measuring plant response. Each treatment was replicated four times.

The results, arranged in table 2, showed that all treatments produced significant inhibition of growth compared with the control. The spray was most effective when applied in a volume of 10–20 ml. per square yard and a concentration of 125–250 p.p.m. There was no significant difference in effect between these two volume-concentration rates or between treatments at 40 and 100 ml. per square yard. The latter may be considered a spray of "saturation" volume. However, the 20 ml. per square yard rate was more effective than 40 ml., 10 ml. more effective than 4 ml., and 4 ml. more effective than 2 ml. (fig. 1).

Growth inhibition was progressively greater as concentration was increased, and volume decreased from saturation levels to 10–20 ml. per square yard, but there was a rapid decrease in effectiveness of the spray with further reductions in volume when the amount of compound was kept constant (fig. 2).

TABLE 2

EFFECT OF AQUEOUS SPRAYS OF THE AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED TO KIDNEY BEANS AT 2.5 MG. PER SQUARE YARD IN DIFFERENT VOLUME-CONCENTRATION RATES

SPRAY VOL. (ML. PER SQUARE YARD)	CONCENTRATION		GREEN WEIGHT OF LEAVES OF NEW GROWTH*	
	(Per cent)	(P.p.m.)	Setting 2 (gm.)	Setting 3 (gm.)
Control........	0	0	2.55	2.55
100.............	0.0025	25	1.35	1.82
40.............	0.00625	62.5	1.35	1.42
20.............	0.0125	125	0.59	0.72
10.............	0.0250	250	0.64	0.40
4.............	0.0625	625	1.82	0.89
2.............	0.1250	1250	†	1.74

* Minimum significant difference between means at 5% level of probability is 0.42 gm.
† Volume rate of 2 ml. per square yard could not be delivered at setting 2.
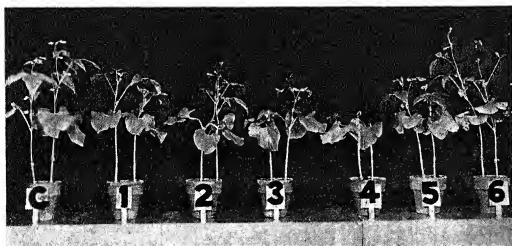
FIG. 1.—Effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in different volume-concentration rates on kidney beans. Setting 3 of the spray gun. C, control; 1, 100 ml. per square yard; 2, 40 ml. per square yard; 3, 20 ml. per square yard; 4, 10 ml. per square yard; 5, 4 ml. per square yard; 6, 2 ml. per square yard.

Further experiments were conducted at low-volume rates in view of the following considerations. Previous work on weed-killing, because of the necessity of getting complete coverage of plants to produce contact injury, has been carried out at high-volume rates, i.e., 100-150 gallons per acre, which is roughly equivalent to 100 ml. per square yard. Compounds of the phenoxyacetic acid type are translocated and systemic in effect and may therefore be effective at far lower-volume rates. Even single droplets will kill young plants. Conceivably, weed-killing could be accomplished by sprays distributed from airplanes if low-volume rates were found to be acceptable. The results reported above indicated that plant responses to low-volume sprays were substantially different in magnitude from those obtained when applications were made in larger volumes.
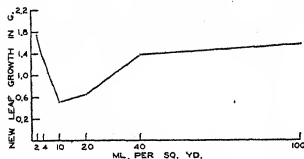
FIG. 2.—Effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied to kidney beans at a constant rate of 2.5 mg. per square yard in different volumes.

FIG. 3.—Effect of four different concentrations of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in four low-volume spray rates on soybeans.

An experiment was set up in which four different concentrations of an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid were each sprayed at four different volume rates, namely, 2, 4, 6, and 8 ml. per square yard. Soybean plants with three well-formed trifoliate leaves were used as experimental material, and similar treatments were applied to young tomato plants. Weights of the leaves of the entire plant, 20 days after spraying, were used as the criterion for measuring response.

The results are shown in table 3, arranged graphically in figure 3, and illustrated by figures 4 and 5.

In evaluating the graph (fig. 3) it can

TABLE 3

EFFECT OF LOW-VOLUME SPRAYS OF THE AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SOYBEANS

Volume rate (ml. per square yard)	Concentration (per cent)	Weight rate (mg. per square yard)	Leaf weight (gm.)*
2.............	0.25	5	1.25
	0.70	14	1.28
	1.40	28	1.24
	3.00	60	0.45
4.............	0.25	10	0.89
	0.70	28	0.60
	1.40	56	0.21
	3.00	120	0.15
6.............	0.25	15	0.50
	0.70	42	0.33
	1.40	84	0.24
	3.00	180	0.15
8.............	0.25	20	0.24
	0.70	56	0.32
	1.40	112	0.20
	3.00	240	0.14

* Minimum significant difference between means at 5% level of probability is 0.38 gm.

safely be assumed that where the green
weight of leaves was 1.2 gm. or more,
there was no inhibitory effect, and where
the weight was 0.45 gm. or less, complete
inhibition or "kill" had been effected.

Similar results were obtained with to-
mato plants, except that distortion was

pound; but a 3 per cent application (60
mg. per square yard) caused complete
inhibition. (2) At a 4-ml. per square yard
volume rate, a spray of 1.4 per cent con-
centration (56 mg. per square yard)
killed soybeans. A concentration of 0.70
per cent was not sufficiently strong.
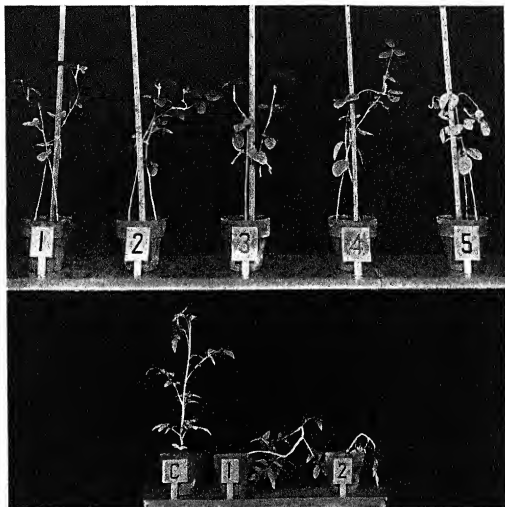
FIGS. 4, 5.—Fig. 4, effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied at a
volume rate of 2 ml. per square yard on soybeans, 14 days after treatment. *1*, 0.25%; *2*, 0.35%; *3*, 0.70%;
4, 1.40%; *5*, 3.00%. Only the treatment at 3% was effective in inhibiting growth. Fig. 5, effect of sprays of
the ammonium salt of 2,4-dichlorophenoxyacetic acid applied at a rate of 14 mg. per square yard on tomatoes.
C, control (av. wt., 6.13 gm.); *1*, 2 ml. per square yard, 0.70% (av. wt., 4.26 gm.); *2*, 4 ml. per square yard,
0.35% (av. wt., 1.64 gm.). Note greater effectiveness of the same amount of compound applied in the larger
volume.

accomplished by lighter dosages and the
data were less consistent.

The following conclusions were ten-
able: (1) At a volume rate of 2 ml. per
square yard there was no inhibition in
growth from spray applications of 0.25–
1.40 per cent concentration of com-

(3) In a spray volume of 6 ml. per square
yard a concentration of 0.70 per cent (42
mg. per square yard) killed soybeans.
At this same volume rate a concentration
of 0.25 per cent was not sufficient to
cause complete inhibition of soybeans
but was highly effective on tomatoes.

(4) Applied in a volume of 8 ml. per square yard at a concentration of 0.25 per cent (20 mg. per square yard), the spray killed both soybeans and tomatoes.

These results showed that in sprays of low-volume rate, as the volume was reduced, progressively more growth-inhibiting substance was required to produce the same response. At least 60 mg. per square yard of the ammonium salt of 2,4-dichlorophenoxyacetic acid was needed to kill young soybean plants with sprays applied at a 2-ml. per square yard rate, and only 20 mg. per square yard at an 8-ml. per square yard volume rate.

DROPLET SIZE STUDIES

To study the relative effectiveness of 2,4-dichlorophenoxyacetic acid when applied in aqueous sprays of different droplet size, two settings (no. 1 and no. 4, table 1) of the spray gun were used. When young kidney-bean plants were treated with large- and with small-droplet sprays at three volume rates and three rates of compound, it was found that those composed of the larger droplets were more effective (table 4 and fig. 6). This result was due mostly to a higher percentage of spray interception when the large droplets were used.

The sprays of small-droplet size were found to be more effective when applied in a relatively large volume, such as 60 ml. per square yard (table 4 and fig. 6). The relative ineffectiveness of small-droplet sprays at low volume might be due to the fact that many of the droplets were left "stranded" on islets on the lamina of the leaf without access to conductive tissue. In sprays of large volume there would be a coalescence of droplets into larger ones (as in large-droplet sprays at low volume), which would be more likely to come in contact at some

point with conductive tissue, so that all the compound in the drop could be absorbed and transported.

INTERCEPTION STUDIES

Experiments were carried out to investigate possible relationships between amount of interception of sprays and differences in droplet size and volume rate

TABLE 4

EFFECT OF LARGE AND SMALL-DROPLET AQUEOUS SPRAYS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH OF KIDNEY BEANS HARVESTED 12 DAYS AFTER APPLICATION

VOLUME RATE (ML. PER SQUARE YARD)	WEIGHT RATE (MG. PER SQUARE YARD)	GREEN WEIGHT OF NEW GROWTH*	
		Large drops (gm.)	Small drops (gm.)
60............	0.5	5.76	5.36
	1.0	2.88	5.19
	2.0	0.93	4.10
30............	0.5	2.89	6.02
	1.0	2.58	5.40
	2.0	0.99	5.28
10............	0.5	3.46	6.22
	1.0	2.14	6.79
	2.0	1.16	5.62
Control.......	0.0	7.76	7.76

* Minimum significant difference between means at 5% level of probability is 1.61 gm.

and further to correlate the findings with plant response.

In order to obtain a measure of the amount of growth-inhibiting substance intercepted by plants exposed to different types of sprays, a method for determining the percentage of recovery of dye solution, applied under the same conditions, was employed. Two stable water-soluble Du Pont dyes were selected, namely, anthraquinone blue and crocein scarlet. A 1 per cent stock solution and nine dilutions, ranging from 1 to 50 mg. per cent, were made up with each dye,
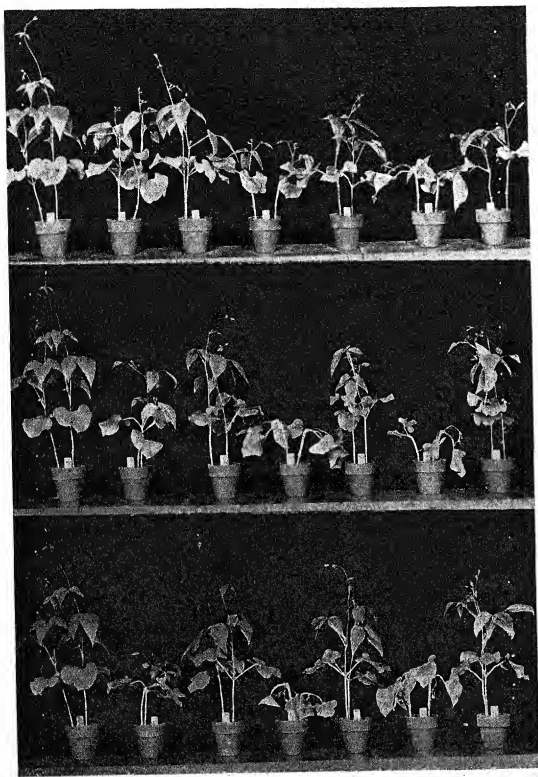
FIG. 6.—Kidney-bean plants treated with 2,4-dichlorophenoxyacetic acid applied in sprays of two different droplet sizes in a volume of 60 ml. per square yard (above), 30 ml. per square yard (center), and 10 ml. per square yard (below). Left to right: Control, 0.5 mg. per square yard (large drops); 0.5 mg. per square yard (small drops); 1.0 mg. per square yard (large drops); 1.0 mg. per square yard (small drops); 2.0 mg. per square yard (large drops); and 2.0 mg. per square yard (small drops).

and standard calibration curves on the Evelyn photoelectric photometer were constructed.

Using three different settings of the gun, the dye solutions were sprayed on soybean plants, the second trifoliate leaves of which were beginning to unfold. The solutions were applied in the same

TABLE 5

INTERCEPTION BY SOYBEAN PLANTS OF DYE SOLUTIONS APPLIED IN DIFFERENT TYPES OF SPRAYS

SPRAY-GUN SETTING NO.	VOL. SOLU-TION SPRAYED (ML.)	PLANT AREA (SQ. IN.)	CON-CEN-TRATION OF WASH (MG. PER CENT)	SOLUTION IN-TERCEPTED OF TOTAL VOLUME SPRAYED	
				Per plant (per cent)	Per square inch (per cent)
1.........	5	10.77	1.70	6.8	0.68
		7.22	0.50	2.0	0.28
		8.24	1.20	4.8	0.58
2.........	5	8.06	1.00	4.0	0.50
		7.80	0.95	3.8	0.49
		8.24	1.25	5.0	0.61
4.........	5	9.42	0.25	1.0	0.11
		7.65	0.20	0.8	0.10
		9.55	0.40	1.6	0.17
4.........	10	9.22	0.80	1.6	0.17
		10.02	0.60	1.2	0.12
		9.25	0.60	1.2	0.13

manner as those sprays containing 2,4-dichlorophenoxyacetic acid. Following the spray treatment, the plants were washed immediately in 200 ml. of distilled water, and the concentrations of dye in the different washes were determined. From these figures the per cent interception per plant was calculated. The approximate surface area of each plant was determined by obtaining the leaf area with a planimeter and the stem area by applying the formula for the

lateral area of a cylinder to measurements of height and mean width. The interception per square inch, in terms of total volume sprayed, was calculated. The results are shown in table 5.

In order to obtain a general figure for the average amount of interception per plant, the average surface area for all plants used in this experiment, calculated to be 8.79 square inches, was multiplied by the average per cent interception per square inch for each spray type. These data are shown in table 6.

TABLE 6

AVERAGE INTERCEPTION AND RETENTION OF DYE SOLUTION BY SOYBEAN PLANTS EX-POSED TO DIFFERENT TYPES OF SPRAY

Spray-gun setting no.	Vol. solution sprayed (ml.)	Average rate of in-terception per square inch (per cent)	Average rate of in-terception per plant (per cent)
1...............	5	0.51	4.48
2...............	5	0.53	4.66
4...............	5	0.13	1.14
4...............	10	0.14	1.23

Only about one-fourth as much spray was intercepted and retained per plant in applications composed of small droplets as in those composed of large droplets. The sprays of large-size (ca. 561 μ diam.) and intermediate (ca. 250 μ diam.) droplets gave essentially the same result, namely, that about 4½ per cent was intercepted per plant. They had in common the characteristic that the drops were large enough to fall directly, whereas the spray of small droplets (ca. 30 μ diam.) remained suspended in the air for a while, and some may have been diverted to additional surfaces of the chamber.

Some preliminary experiments were performed to study the relation of spray

volume to degree of interception, using setting 3 of the spray gun and young kidney beans for plant material. The data were corrected for differences in plant size. It was found that, at a volume rate of 100 ml. per square yard, 2.07 per cent of the spray was intercepted per plant; at 10 ml. per square yard, 3.19 per cent; and at 2 ml. per square yard, 1.94 per cent. This provided evidence that some of the differences in plant response correlated with volume of spray (see table 2) could be attributed to a difference in the amount of spray intercepted and retained.

Summary

1. An apparatus was set up, with which sprays of different droplet size could be applied at desired volume rates on plants for the purpose of determining the effect of growth-regulating compounds applied as herbicides.

2. Aqueous sprays, containing a constant threshold amount of the ammonium salt of 2,4-dichlorophenoxyacetic acid, were found to be most effective at a volume rate of 10–20 ml. per square yard when applied to young kidney-bean plants. Rates of 40–100 ml. per square yard were less effective, and there was a progressive decrease in magnitude of response as the volume was reduced below 10 ml. per square yard.

3. Further studies were made on sprays applied at the low rates of 8, 6, 4,

and 2 ml. per square yard. It was shown that, as the volume was reduced, a progressively larger amount of growth-inhibiting substance was required to kill young soybean plants. Twenty mg. of compound per square yard was needed at the 8-ml. per square yard volume rate and at least 60 mg. per square yard (3 per cent concentration) at the 2-ml. per square yard rate.

4. Sprays of relatively large-droplet size (561–250 μ av. diam.) were more effective than those of smaller-droplet size (30 μ av. diam.).

5. The investigations on interception showed that more solution was deposited and retained on a plant when sprays of larger-droplet size were used. There was also an indication that a higher percentage of the spray was intercepted and retained when applied at volume rates of 10–20-ml. per square yard than in smaller or larger volumes.

6. For most efficient use of growth-regulating compounds, they should be applied in sprays devoid of finely atomized particles and in volumes of 10–20 ml. per square yard rather than at saturation levels of about 100 ml. per square yard. Rates below 10 ml. per square yard, while less efficient in terms of amount of compound required, are of interest. The fact that growth inhibitors can be effective when applied in such low volumes opens up new fields for large-scale practical application of herbicides.

THE RESPONSE OF KIDNEY-BEAN AND SOYBEAN PLANTS TO AQUEOUS-SPRAY APPLICATIONS OF 2,4-DICHLOROPHE-NOXYACETIC ACID WITH AND WITHOUT CARBOWAX[1]

W. B. ENNIS, JR., LT., U.S.N.R., AND F. T. BOYD, CAPT., A.U.S.

Introduction

It has been reported (3) from single-droplet tests on kidney-bean plants that Carbowax not only enhanced the inhibitory action of 2,4-dichlorophenoxyacetic acid but also appeared to possess deleterious properties of its own when applied in aqueous sprays. These investigations were undertaken to determine whether the addition of Carbowax to aqueous solutions of 2,4-dichlorophenoxyacetic acid would increase their herbicidal effectiveness when sprayed in relatively low volumes on soybean and kidney-bean plants.

Materials and methods

For the purpose of this investigation three polyethylene glycols, which are commercially available as Carbowax 1500, 1540, and 4000, were used. MITCHELL and HAMNER have discussed the properties of these compounds (3).

PLANT MATERIAL.—Illini soybean and red kidney-bean plants were grown in 4-inch pots containing a fertile greenhouse-soil mixture and were thinned to two uniform plants per pot. When treated, the soybean plants were 17 days old and the red kidney-bean plants 13 days old. Care was exercised in selecting uniform plants for the experiment. The plants were harvested 11 days following spray application.

SOLUTIONS AND METHODS OF APPLICATION.—Thirty ml. aqueous solutions

containing 1, 5, and 10 mg., respectively, of 2,4-dichlorophenoxyacetic acid, and similar solutions, to which 0.5% Carbowax 1500, 1540, and 4000 had been added, were used on the soybean plants; for treatment of kidney-bean plants the aqueous and the 0.5% Carbowax solutions contained 0.5, 2.5, and 5.0 mg. per 30 ml. The solutions containing Carbowax were prepared in a manner similar to that described by MITCHELL and HAMNER (3). These concentrations had previously been found to approach threshold concentrations for the test plant at the stage of development indicated above. Both soybean and kidney-bean plants were treated with 0.5% aqueous solutions of the three Carbowaxes for controls.

All solutions were applied at the rate of 30 ml. per square yard with a DeVilbiss paint-spray-gun apparatus under 20 pounds per square inch constant air pressure, which produces a medium-size droplet spray. A 14×14-inch spray chamber was employed, and each of the four pots of plants used in one test was sprayed singly.

CRITERIA OF GROWTH.—A few hours prior to treatment, individual plant measurements were taken of all plants by measuring the length of stem between unifoliate leaf node and node of the youngest trifoliate leaf. The same measurement was taken just prior to harvest, and the difference in these two figures was taken as one measure of growth. Green and dry weights of the plant portion above the unifoliate leaves were also taken as additional criteria of effect.

Observations

In the case of kidney-bean plants, increasing degrees of epinasty resulted when increasing amounts of 2,4-dichlorophenoxyacetic acid were applied (fig. 1). When the acid was applied in aqueous solutions containing either 0.5% Carbowax 1500, 1540, or 4000, a greater degree of stem curvature was produced, indicative of a greater degree of plant inhibition, than from equal rates of acid applied in a wholly aqueous spray (fig. 2). Similar effects were obtained by MITCHELL and HAMNER (3) when the droplet test was employed.

On the other hand, the response of soybean plants to the Carbowax sprays differed considerably from that of kidney beans. In figures 2 and 3 it is to be noted that the wholly aqueous sprays of 2,4-dichlorophenoxyacetic acid produced as much epinasty as the corresponding
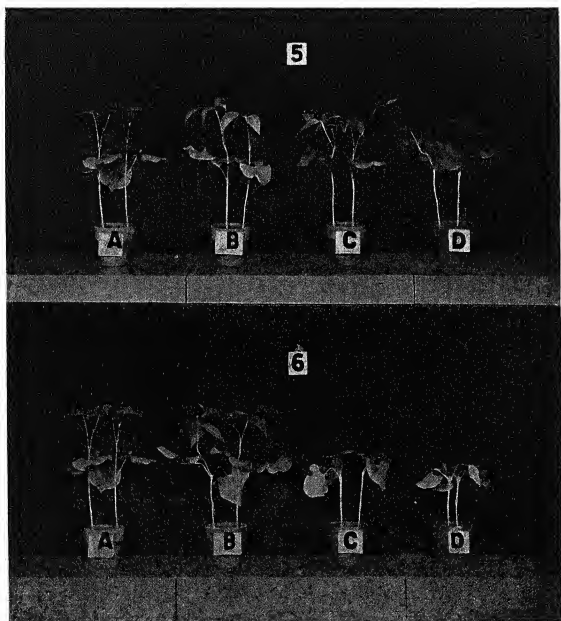
Fig. 1.—Effects of spraying kidney-bean plants with 2,4-dichlorophenoxyacetic acid in wholly aqueous solutions (5, above) and 0.5% Carbowax 1500 solutions (6, below). The volume of spray was 30 ml. per square yard and was applied 13 days after planting. Acid rate per square yard: *A*, carrier control; *B*, 0.5 mg.; *C*, 2.5 mg.; *D*, 5.0 mg. Eleven days after treatment.
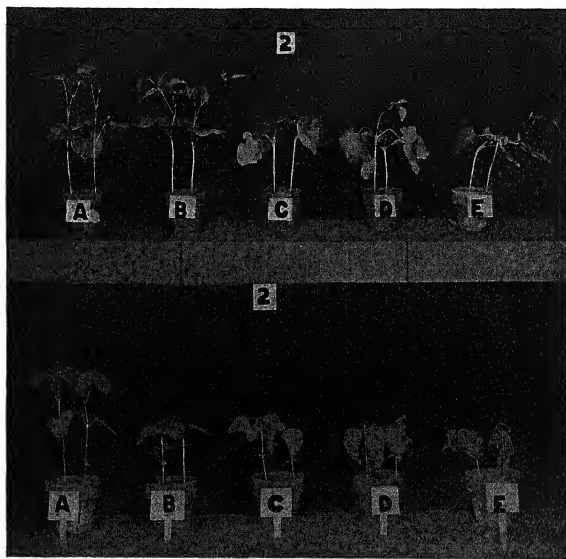
Fig. 2.—Effects of spraying kidney-bean (above) and soybean plants (below) with 2,4-dichlorophenoxyacetic acid in wholly aqueous solution and in 0.5% Carbowax solutions. The acid was applied in 30-ml. spray solutions at the rate of 5.0 and 2.5 mg. per square yard, respectively, to the soybeans and kidney beans. A, untreated; B, water; C, Carbowax 1500; D, Carbowax 1540; E, Carbowax 4000. Eleven days after treatment.
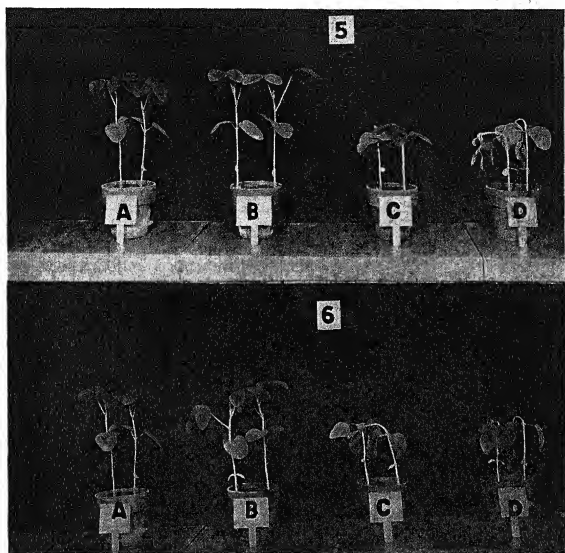
Fig. 3.—Effects of spraying soybean plants with 2,4-dichlorophenoxyacetic acid in wholly aqueous solutions (5, above) and 0.5% Carbowax 1500 solutions (6, below). The volume of spray was 30 ml. per square yard and was applied 17 days after planting. Acid rate per square yard: *A*, control; *B*, 1.0 mg.; *C*, 2.5 mg.; *D*, 10.0 mg. Eleven days after treatment.
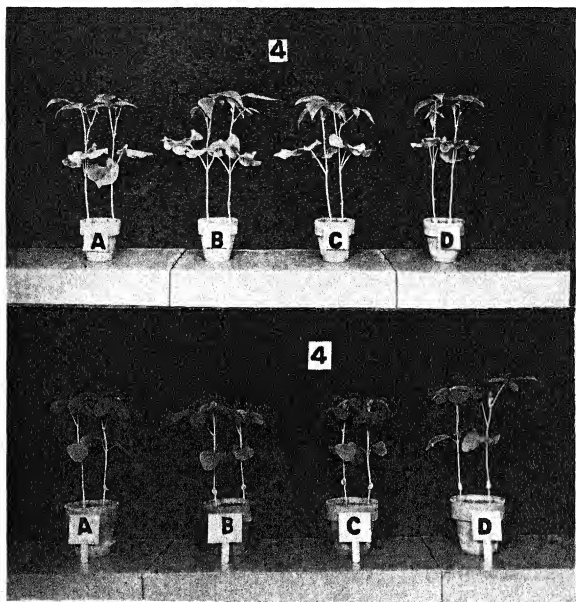
FIG. 4.—Effects of spraying kidney-bean (above) and soybean plants (below) with 0.5% Carbowax solutions at a volume rate of 30 ml. per square yard (each treated at same age as indicated in figs. 1 and 3). *A*, control; *B*, Carbowax 1500; *C*, Carbowax 1540; *D*, Carbowax 4000. Eleven days after treatment.

treatments involving either of the Carbo-waxes. Spray applications of 0.5% solutions of the three waxes did not produce any noticeable response in the soybean or kidney-bean plants (fig. 4).

The three criteria of treatment responses were in surprisingly close agreement. It is apparent (tables 1, 2, and 3) that the compound was significantly more effective in producing inhibition of growth in kidney-bean plants when applied at the rate of 2.5 mg. per square

TABLE 1

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON STEM ELONGATION OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN STEM LENGTHS (CM.) BETWEEN UNIFOLIATE LEAF NODE AND YOUNGEST TRIFOLIATE LEAF NODE OF EIGHT PLANTS. FINAL MEASUREMENTS WERE MADE 11 DAYS AFTER TREATMENT*

2,4-DICHLORO-PHENOXYACETIC ACID (MG. PER SQUARE YARD)	CARRIER SOLUTIONS			
	Aqueous	0.5% Carbo-wax 1500	0.5% Carbo-wax 1540	0.5% Carbo-wax 4000
	Soybeans			
0.0 (control)...	6.1	6.8	7.5	7.3
1.0...........	6.4	6.5	5.5	3.8
5.0...........	3.9	3.7	3.3	3.1
10.0...........	2.8	2.9	3.3	2.4
	Kidney beans			
0.0 (control)...	14.7	14.5	12.8	11.3
0.5...........	14.6	12.8	14.1	11.1
2.5...........	10.6	5.0	6.1	5.7
5.0...........	8.2	4.1	5.1	4.2

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.9 cm., at the 1% level of probability, 1.2 cm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 2.2 cm., at the 1% level of probability, 2.9 cm.

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE GREEN WEIGHT OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN GREEN WEIGHTS (GM.) OF VEGETATIVE PORTION ABOVE THE UNIFOLIATE LEAVES OF EIGHT PLANTS*

2,4-DICHLORO-PHENOXYACETIC ACID (MG. PER SQUARE YARD)	CARRIER SOLUTIONS			
	Aqueous	0.5% Carbo-wax 1500	0.5% Carbo-wax 1540	0.5% Carbo-wax 4000
	Soybeans			
0.0 (control)...	3.58	3.74	3.90	3.78
1.0...........	3.51	3.38	2.99	2.25
5.0...........	2.34	2.30	2.49	2.11
10.0...........	2.19	2.28	2.18	1.80
	Kidney beans			
0.0 (control)...	7.08	7.52	7.09	7.09
0.5...........	7.40	6.85	6.88	5.80
2.5...........	5.55	2.45	2.04	2.25
5.0...........	3.71	1.49	1.39	1.45

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.61 gm., at the 1% level of probability, 0.82 gm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 1.25 gm., at the 1% level of probability, 1.67 gm.

yard in Carbowax solutions than when applied in wholly aqueous solutions. When the compound was applied to soybean plants at a rate of 5 and 10 mg. per square yard, no significant difference in the inhibition of growth was produced by the Carbowax sprays, as compared to wholly aqueous sprays (tables 1, 2, and 3).

The general responses of various plant portions of soybean and kidney-bean plants to the acid were essentially the same as have already been described (2, 3).

Discussion

It has previously been shown (3) that solutions containing above 9% Carbowax 1500 were toxic to greenhouse-grown tomato plants and that soybean and kidney-bean leaves became yellow-

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE OVEN-DRY WEIGHT OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN FRESH WEIGHTS (GM.) OF THE VEGETATIVE PORTION ABOVE THE UNIFOLIATE LEAVES OF EIGHT PLANTS*

2,4-DICHLORO-PHENOXYACETIC ACID (MG. PER SQUARE YARD)	CARRIER SOLUTIONS			
	Aqueous	0.5% Carbo-wax 1500	0.5% Carbo-wax 1540	0.5% Carbo-wax 4000
	Soybeans			
0.0 (control)...	0.80	0.79	0.85	0.81
1.0............	0.76	0.70	0.64	0.45
5.0............	0.42	0.38	0.39	0.34
10.0............	0.32	0.34	0.34	0.26
	Kidney beans			
0.0 (control)...	1.20	1.22	1.16	1.19
0.5............	1.05	0.96	0.98	0.87
2.5............	0.72	0.28	0.23	0.31
5.0............	0.43	0.15	0.15	0.18

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.12 gm., at the 1% level of probability, 0.16 gm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 0.18 gm., at the 1% level of probability, 0.24 gm.

ish when sprayed with solutions containing 10 and 15% by weight of Carbowax 1500, while lower concentrations of Carbowax did not appear to produce any deleterious effects. Extensive exploratory studies with soybeans have largely corroborated these results. While investigating Carbowaxes in this study it was found that the 1-mg. applications of 2,4-dichlorophenoxyacetic acid appeared consistently to produce greater inhibition of growth of soybeans when applied in 0.5% Carbowax 4000 than in a wholly aqueous spray or in either 0.5% Carbowax 1500 or 1540 (tables 1, 2, and 3). This would suggest that Carbowax 4000 was more effective in some way in accentuating the action of the growth-regulating substance than were Carbowax 1500 or 1540.

The 2,4-dichlorophenoxyacetic acid produced more highly significant inhibition of growth in kidney-bean plants when sprayed in a solution containing 0.5% of Carbowax 1500, 1540, or 4000 than when applied in a wholly aqueous spray. On the other hand, there was a striking difference in the response of soybean plants which were treated similarly. Excepting the 1-mg. applications of 2,4-dichlorophenoxyacetic acid in 0.5% Carbowax 4000 solution, soybean plants sprayed with the compound in Carbowax solutions were not inhibited in growth any more than the plants treated with wholly aqueous solutions.

Since soybean and kidney-bean plants differ markedly in the degree of leaf pubescence, it is suggested that the dissimilarity in the response of these plants to aqueous-Carbowax spray application of 2,4-dichlorophenoxyacetic acid may be attributed to this morphological difference. It appears plausible that the compound may be readily absorbed by the leaf hairs of soybeans, whereas the more glabrous leaves of kidney beans perhaps do not absorb the wholly aqueous spray so rapidly and the presence of Carbowax effects the retention of moisture on the leaf surface for an interval sufficient to permit absorption of the compound by the epidermal leaf tissues. Further study will be necessary to determine how and

in what form the substance enters the plant, before this differential response can be fully explained.

The value of Carbowax as a co-solvent for increasing the concentration of 2,4-dichlorophenoxyacetic acid in aqueous solutions has been suggested (1). ZIMMERMAN (4) has found that, in general, salts, esters, and amides are approximately equal in activity to the acids from which they are derived. This has been borne out in somewhat extensive studies, not reported here, which have indicated that, for spraying many types of broadleaf plants, the ammonium salt form of the acid is not statistically different from the acid in its effectiveness as a plant growth-regulator. Many of the salts, such as the ammonium and sodium salt, are directly soluble in water. Considering that it requires 4.5 parts by weight of Carbowax 1500 to prepare a solution containing 1 part by weight of 2,4-dichlorophenoxyacetic acid (1), the proportional amount of Carbowax required to prepare concentrations equal to that of the directly water-soluble ammonium salt is large and relatively expensive. The results of this study indicate that Carbowax offers no advantage

for increasing the inhibitory action of 2,4-dichlorophenoxyacetic acid upon the soybean. Since there are many broadleaf plants that have morphological characteristics similar to the soybean, the general use of Carbowax as a co-solvent may not be justified in herbicidal sprays. It is suggested that attention be given to the use of the directly water-soluble ammonium salt or other water-soluble salt form of 2,4-dichlorophenoxyacetic acid for general herbicidal-spray purposes.

Summary

1. Soybean plants differ from kidney-bean plants in their response to aqueous-spray applications of 2,4-dichlorophenoxyacetic acid containing Carbowax.

2. The addition of any of three Carbowaxes to aqueous-spray solutions of 2,4-dichlorophenoxyacetic acid enhanced its activity in inhibiting growth of kidney-bean plants but did not aid in inducing inhibitory responses in soybean plants.

3. Because of its solubility properties, the ammonium salt of 2,4-dichlorophenoxyacetic acid is suggested instead of the acid form for use in general herbicidal sprays.

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TWO METHODS FOR THE DETERMINATION OF THE HERBICIDAL EFFECTIVENESS OF PLANT GROWTH-REGULATING SUBSTANCES IN OIL SOLUTION ON BROADLEAF PLANTS[1]

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Introduction

Oil solutions of plant growth-regulating substances applied as herbicides possess several advantages over aqueous solutions. In the first place, the oil solutions generally produce a greater inhibitory effect per unit of compound, probably because of their lower rate of evaporation and the more rapid entry of the agent into the plant tissues as a result of the ready penetration of the leaf cuticle by such solutions. Second, rainfall, if occurring shortly after exposure of the plant to the agent, severely reduces the effectiveness of aqueous, but not of oil, sprays (4). Lastly, the ready miscibility in oil of various esters of the phenoxyacetic acid series and the relative ease with which many such herbicidal agents may be dissolved in tributylphosphate and oil (1), permit high concentrations of the agent to be obtained. Such highly concentrated oil sprays have been shown to be most effective at very low volume rates of application. On the other hand, the solubility of the phenoxyacetic acids and their salts in aqueous solution is, in general, of a rather low order.

To test the effectiveness of various herbicidal agents in oil, it was first necessary to develop adequate testing methods, two of which are described below. Aside from any specificity of action which a herbicidal compound may exhibit on a particular plant species, these methods

have been found satisfactory for the testing of many hundreds of compounds.

Experimental

THE OIL-DROPLET TEST

This test is a modification of the water-droplet test of MITCHELL and HAMNER (2). Kidney beans were used as test plants, and the herbicidal compounds were applied when the primary leaves had expanded and the second internode was 1 inch in length. The compounds were put into solution and tested in the following manner: 50 mg. were dissolved in 0.2 or 0.4 ml. of tributylphosphate, and a sufficient amount of no. 2 fuel oil (kerosene base) was added to bring the total volume to 10.0 ml. If 50 mg. of the compound were not soluble in 0.4 ml. of tributylphosphate, the compound was arbitrarily considered insoluble. After a thorough shaking, the solution was allowed to stand for a day to determine whether precipitation occurred. Of this stock solution, 1.0 ml. was diluted to 10 ml. with fuel oil to give a 500 p.p.m. solution. Six kidney-bean plants were then treated by placing a single drop of solution (0.01 ml.), containing 5γ of agent, on the base of the blade of one of the two primary leaves of each plant. A $\frac{1}{4}$-ml. tuberculin syringe was used to give uniform droplet size. The plants were allowed to grow for 10 days, after which all growth above the primary leaves was removed and fresh weights were obtained. Oil and tributylphosphate-oil controls were used with each group of

compounds to be tested. The data were not statistically analyzed.

THE OIL-SPRAY TEST

This was designed to test for small differences in effectiveness, based on a low amount of agent and small volume of solution applied at sublethal levels. Soybean plants were used because of their uniformity of growth and were exposed when the second trifoliate leaf was fully expanded. Four pots of three plants each were sprayed. Data from the results were then statistically analyzed.

The solutions of compounds to be tested were obtained by adding 1 ml. of the 0.5% stock solution (see "Oil-Droplet Test," above) to 1.5 ml. of fuel oil. This, when sprayed in a chamber with a base area of $\frac{1}{2}$ square yard, gave an application of 10 mg. of agent in a 5-ml. volume per square yard. The solutions were applied with an atomizer attached to a laboratory air jet at approximately 15 pounds pressure. The plants were then allowed to grow for 21 days under normal greenhouse conditions, at the end of which time all growth above the second trifoliate leaves was removed and fresh weights were taken. Oil and tributylphosphate-oil controls, using the same volume sprays, were used with each group of compounds tested.

Discussion

These two tests, both employing growth-inhibition as a criterion of effect, provide ready means for evaluating the herbicidal effectiveness of many compounds in oil solution. It was necessary to supplement the oil-droplet test, which is principally a preliminary screening procedure for the determination of the better herbicidal compounds, with the oil-spray test, because of variable data obtained from the former. This varia-

bility depends primarily on the angle of attachment of the primary leaf on which the oil drop is placed, the angle governing the amount of oil, and hence the amount of agent, which will spread down the petiole and reach the base of the second internode. On the other hand, the water-droplet test of MITCHELL and HAMNER (2) and the corn-germination test (3), judging from the results obtained in these laboratories, appear to be thoroughly satisfactory for accurately evaluating the action of herbicidal agents in aqueous solution. The aqueous insolubility of many compounds, however, and the practicability of using herbicidal oil sprays require the use of some method of testing compounds in oil solutions. Despite the variability of the oil-droplet test, it is sufficiently accurate to be of value for preliminary screening, when one considers the ease and rapidity with which many growth-regulating substances may be tested. The definitely poorer compounds can be discarded and the better ones further screened by the oil-spray test. A comparison of some of the better phenoxyacetic acid derivatives tested by these two methods is given in table 1. It will be apparent from the data that, in oil solutions, derivatives of 2,4,5-trichlorophenoxyacetic acid produce a greater inhibitory effect in general than either 4-chloro derivatives or the 2,4-dichlorophenoxyacetic acid.

Summary

Two tests are described which permit an evaluation of the herbicidal effectiveness on broadleaf plants of growth-regulating substances dissolved either in oil alone or in tributylphosphate and oil. The first, the oil-droplet test, is less reliable than the oil-spray test, but its value lies in the ease and rapidity with which a large number of substances may

be tested. The second test, based on low rates and volumes of application, is designed to test for small differences, and it has proved very accurate. Both tests use growth inhibition as a criterion of herbicidal effectiveness.

TABLE 1

COMPARISON OF THE FRESH WEIGHTS (EXPRESSED IN % OF CONTROL) OF PLANTS
TREATED WITH VARIOUS HERBICIDAL COMPOUNDS BY THE
OIL-DROPLET AND OIL-SPRAY TESTS

Compound	Oil-droplet test	Oil-spray test
Untreated control....................................	100	100
4-chlorophenoxyacetic acid............................	48	40
4-chlorophenoxyacetyl chloride........................	54	52
4-chlorophenoxyacetic phenylhydrazide.................	26	23
Beta-chloroethyl 4-chlorophenoxyacetate...............	12	40
2-methyl, 4-chlorophenoxyacetic acid..................	70	45
2,4-dichlorophenoxyacetic acid........................	41	38
2,4-dichlorophenoxyacetic acid*.......................	95	70
Beta-bromoethyl 2,4-dichlorophenoxyacetate............	9	48
Beta-chloroethyl 2,4-dichlorophenoxyacetate...........	20	51
Isoamyl 2,4-dichlorophenoxyacetate....................	45	56
Trichloroethyl 2,4-dibromophenoxyacetate..............	42	55
Ethyl 2,4-dichlorophenoxyacetate......................	53	44
Beta-bromethyl 2,4-dichlorophenoxyacetate.............	17	51
Butyl 2,4-dichlorophenoxyacetate......................	35	46
2,4,5-trichlorophenoxyacetic acid.....................	22	14
2,4,5-trichlorophenoxyacetyl chloride.................	34	20
2,4,5-trichlorophenoxyacetic 2′,4′-dimethylanilide....	11	13
2,4,5-trichlorophenoxyacetic 2′,4′,6′-trichloroanilide	16	18
2,4,5-trichlorophenoxyacetic meta-chloroanilide.......	13	18
2,4,5-trichlorophenoxyacetic para-anisidide..........	50	27
2,4,5-trichlorophenoxyacetic beta-naphthanilide......	16	9
2,4,5-trichlorophenoxyacetic para-bromanilide........	14	42
2,4,5-trichlorophenoxyacetic di(beta-hydroxy-ethyl)amide	27	22

* Dissolved in water.

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EFFECT OF PLANT GROWTH-REGULATORS IN RELATION TO STAGES OF DEVELOPMENT OF CERTAIN DICOTYLEDONOUS PLANTS[1]

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Introduction

Before an effective program of weed control in dicotyledonous crops can be developed, the responses of the various crop species to different rates and methods of application of chemical herbicides must be known at all stages of development. In the absence of selective herbicides which will kill one species of broad-leaf plant but not others, a differential susceptibility between crop and weed, depending upon stage of development, may possibly provide an avenue of approach to weed control.

Preliminary greenhouse studies showed that, in general, the responsiveness of a plant to herbicidal applications of plant growth-regulators decreased with increasing age when fresh or dry weight of the aerial portion of the plant was taken as the criterion of effectiveness of the treatment. However, only in such crops as lettuce, cabbage, broccoli, cauliflower, etc., are the aerial portions used for food. In other crops either the fruit, the seed, or some underground portion of the plant is used for edible purposes. It was the purpose of this investigation to determine at which stage of development each of a variety of crops is responsive to such chemical herbicides and to compare their responsiveness with the responsiveness of such vegetative crops as cabbage.

Methods

Applications of the ammonium salt of 2,4-dichlorophenoxyacetic acid in aqueous solution were made by spraying. Field spraying was done on relatively windless days, with the plots to be treated inclosed by canvas screens 4 feet high to prevent drifting of the spray. A De Vilbiss paint sprayer, with no. 92 detachable nozzle, was used at 10 pounds of pressure, the air supply being derived from a portable cylinder. With this apparatus and pressure, the spray consisted of relatively large droplets, which settled quickly. A volume of 10.0 ml. of spray per square yard of treated area was used throughout the experiments. Adequate spray coverage could be readily achieved at this volume.

The ammonium salt of 2,4-dichlorophenoxyacetic acid was employed instead of the acid itself, since concentrations of the salt up to 2.9% can be obtained in water. When higher concentrations were required, sufficient diethylamine was added to dissolve the acid.

The usual agronomic practices were carried out in growing the plant materials.

Experimental results

CABBAGE

Single plots of four 10-foot rows of Jersey Wakefield cabbage were treated at three stages of development and at rates of 0.03 and 0.3 gm. of ammonium

2,4-dichlorophenoxyacetate per square yard. This corresponds closely to rates of 0.3 and 3.0 pounds of the compound per acre. Fresh weights of heads were taken approximately 1 month after the last spray application (table 1).

TABLE 1

FRESH WEIGHT (POUNDS) OF CABBAGE HEADS PER PLOT TREATED WITH AQUEOUS-SPRAY APPLICATIONS OF AMMONIUM 2,4-DICHLORO-PHENOXYACETATE

RATE (GM. PER SQUARE YARD)	STAGE WHEN TREATED		
	6–8 leaves	Beginning to head	Heads 4 inches in diameter
0.03	0.0	0.0	15.5
0.30	0.0	0.0	13.0
Control (untreated)	17.0		

TABLE 2

MEAN AIR-DRY WEIGHT (GRAMS) OF THRESHED SOYBEANS PER PLOT TREATED WITH AQUEOUS-SPRAY APPLICATIONS OF AMMONIUM 2,4-DI-CHLOROPHENOXYACETATE AT A RATE OF 0.5 POUND PER ACRE. FOUR REPLICATES*

Stage when treated	Average weight of beans per plot
3 inches	135.5
6–8 inches high	145.5
Early flowering	72.0
Early pod	22.8
Control	161.7

* Minimum difference between means for significance at 5% level of probability is 69.3 gm.; at 1% level it is 95.8 gm.

Cabbage was more susceptible to the herbicide in the early stages of growth than it was after the heads had begun to form. At the two earlier stages even the lower rate of application proved rapidly lethal. A relatively mature stage was quite resistant to this herbicide, little inhibition of growth being achieved at the rates used when the heads were over

3 inches in diameter. This trend of responses was similar to that found in greenhouse spray experiments on a variety of plants, when inhibition of the aerial portion of the plant was used as a criterion of the effectiveness of the herbicide.

SOYBEANS

Soybeans, variety Chief, were drilled on May 2, 1945, in 6 × 9-foot plots and treated at four stages of development with a single rate of 0.5 pound of ammonium 2,4-dichlorophenoxyacetate per acre. The pods were allowed to mature, and the plants were harvested on September 28, 1945. The weights of air-dried threshed beans were recorded (table 2).

The immediate effect of the spray treatments on the plants, particularly the two youngest stages, was striking. All young parts showed considerable stem curvature and leaf epinasty, followed later by cortical splitting. This latter phenomenon was due undoubtedly to cell proliferation and root formation in the phloem-cambial region (3). Older plants, treated at the early pod stage, had attained maximum vegetative growth, and the initial effects were less striking.

When the weight of threshable beans was used as a criterion, the later applications were found to be most effective (table 2), which is the converse of the vegetative responses. The younger stages, while severely retarded and considerably distorted by the spray applications, nonetheless showed a remarkable capacity for recovery, eventually producing a normal yield. Older plants, on the other hand, produced significantly reduced yields, while showing little visible effect following treatment. These results parallel those of GRIGSBY (1), who showed that floral formation and seed set in ragweed

could be severely curtailed by applications of 2,4-dichlorophenoxyacetic acid. Undoubtedly, the developing floral and ovarial structures were as severely affected by this herbicide as was the young vegetative growth. The latter could, however, overcome the initial effects by further vegetative growth, but the former could not, since inhibitory influences in the floral structure generally resulted in sterility.

To determine whether the method of application affects the relative susceptibility at various growth stages, soil treatments were also tried. Soybeans were treated at four stages at rates of 3, 10, and 20 pounds of 2,4-dichlorophenoxyacetic acid per acre. The growth-regulator was impregnated in 10-mesh sawdust or processed as granular material, with Fuller's earth as a diluent to facilitate ease of distribution. The treatment materials were uniformly applied by hand to the soil, and care was exercised to prevent them from coming into direct contact with vegetative portions of the plants. All treatments were made in quadruplicate, with each plot consisting of three rows of drilled soybeans 16 feet long. The beans were harvested on September 28, 1945 (table 3).

In addition to the observation that soil applications were relatively inefficient as compared to spray treatments, it was also apparent that the effect of stage of development was not comparable. Only when the herbicide was applied at planting time was a significant reduction in yield obtained. Established plants were relatively unaffected. The extreme sensitivity of plants, when treatments were made at planting time, was not unexpected, as it has been shown that germinating seeds are very susceptible to small applications of herbicidal growth-regulators (2, 4).

TOMATO

In greenhouse trials young tomato plants appeared to be particularly sensitive to aqueous sprays of halogenated derivatives of the phenoxyacetic acid series at low concentrations. The vegetative growth was readily inhibited, and the

TABLE 3

AVERAGE WEIGHT IN GRAMS PER PLOT (48 ROW FEET) OF THRESHED SOYBEANS TREATED AT FOUR STAGES OF DEVELOPMENT WITH THREE RATES OF 2,4-DICHLOROPHENOXYACETIC ACID IN TWO TYPES OF CARRIERS. FOUR REPLICATES*

POUNDS PER ACRE	STAGE WHEN TREATED			
	Planting time	6–8 inches high	Early flowering	Early pod
Sawdust:				
3............	385.5	499.2	392.5	391.5
10............	170.8	541.0	441.0	388.5
20............	93.8	456.0	336.2	449.8
Granular material:				
3............	560.8	425.5	489.2	459.2
10............	240.2	430.8	379.8	410.0
20............	189.0	400.0	333.5	421.2
Control (untreated)............	439.8	493.5	462.8	501.2

* Minimum difference between means for significance at the 5% level of probability is 139.0 gm.; at 1% level it is 184.1 gm.

fruits, when produced, were small and misshapen. In the field, however, the tomato was somewhat similar to the soybean in its response to aqueous sprays of ammonium 2,4-dichlorophenoxyacetate (table 4).

Tomato plants, in single 12 × 12-foot plots, were treated at three stages of development, and at rates of 0.1 and 1.0 pounds per acre of ammonium 2,4-dichlorophenoxyacetate. The fruits were harvested approximately 1 month after

the final treatments and at a time when the majority of all fruits were well colored.

As with soybeans, young tomato plants, unless killed, showed a considerable capacity for vegetative recovery,

TABLE 4

FRESH WEIGHT IN GRAMS OF TOMATOES TREAT-ED AT THREE STAGES OF DEVELOPMENT WITH AMMONIUM 2,4-DICHLOROPHENOXYACETATE AT RATES OF 0.1 AND 1.0 POUNDS PER ACRE

RATE (POUNDS PER ACRE)	STAGE WHEN TREATED		
	8-12 inches high	Early flower-ing	Early fruiting
0.1..................	3268	o	4315
1.0..................	o*	o	1415
Control.............	6525	....	....

* Plants killed.

TABLE 5

AVERAGE FRESH WEIGHT IN GRAMS OF MATURE SWEET-POTATO TUBERS FROM 9 × 9-FOOT PLOTS TREATED WITH TWO RATES OF AMMO-NIUM 2,4-DICHLOROPHENOXYACETATE AT TWO STAGES OF DEVELOPMENT. FOUR REP-LICATES

POUNDS PER ACRE	STAGE WHEN TREATED	
	Early runner	Vines covering ground
0.25..................	o	733
1.0..................	o	69
Control (untreated).......	5093	....

with only a slightly reduced yield. The fruits appeared normal. When they were sprayed in the early flowering stage, not only were the blossoms present at the time of application destroyed, but all subsequent floral development was curtailed, and no fruit was set. When ap-

plied at the early fruiting stage, a considerable reduction in yield was achieved at the 1 pound per acre rate, with the fruits formed somewhat misshapen and frequently seedless. The 0.1 pound per acre rate had but little effect at this stage, and the fruits were normal. In this respect the tomato differed from the soybean in its response to ammonium 2,4-dichlorophenoxyacetate, since in the soybean the early pod (fruiting) stage was the most sensitive of all stages in respect to fruit set (table 2).

SWEET POTATOES AND SUGAR BEETS

Puerto Rico sweet potatoes and Wanzleben sugar beets were treated to determine the effect of aqueous sprays of ammonium 2,4-dichlorophenoxyacetate on yield of root crops. No yield data were obtained from the plantings of sugar beets because treatments of as little as 0.5 pounds per acre at two stages of development—5-7 inches in height and 12-14 inches in height with roots 4-6 inches in length and thumb-size in diameter—effectively killed all plants.

Sweet potatoes were planted on May 17, 1945, as well-rooted slips spaced 18 inches apart in 3-foot rows. Some plants were treated on July 24, when they were in the early runner stage, and others on August 14, when the vines had completely covered the ground. Plants were sprayed with an aqueous solution of ammonium 2,4-dichlorophenoxyacetate at rates of 0.25 and 1.0 pounds per acre. On October 3, 1945, the potatoes were dug and weighed. The data of table 5 indicate that susceptibility of sweet potatoes to the growth-regulator decreased with increasing age and size of plant. The sweet potato was, however, affected by very low dosages of the compound, being one of the most readily killed of the crop plants studied.

Discussion

The data obtained from herbicidal-spray trials applied at various stages of development showed that the selection of the most susceptible stage of any crop depends on the criterion of effect used. With vegetative growth, either aerial or root, employed as the criterion, the earliest stages of growth, in general, showed the greatest inhibitory response. If fruit set was used for the evaluation of response, the early flowering or early pod stage was the most readily affected period of growth. These differences, however, are readily reconcilable when one considers that in each instance it is immature structures—i.e., immature vegetative growth—or floral parts which respond to the treatments, thus causing inhibitory effects which are reflected in the final yields.

In any weed-control program it will be necessary, therefore, to spray at the proper time if a differential weed-killing is to be achieved, and no one time or rate will be suitable for all crops. Sufficient data are not available to state conclusively which developmental stage is the most susceptible, although it would appear from the soybean spray trials that an application which will effectively prevent seed set when applied at the flowering stage may not kill even the youngest plants.

It is probable that quantities of herbicidal compounds which cause no damage to certain crop plants would be lethal doses for many young or seedling weeds. The experiments in this paper indicate roughly the levels of 2,4-dichlorophenoxyacetic acid that will not injure certain crop plants. Experiments concerned with determination of minimum rates of herbicide necessary to kill weed species at various stages of development would be of value.

Summary and conclusions

1. Five species of field crops (cabbage, soybean, tomato, sweet potato, and sugar beet) at various developmental stages were treated with aqueous-spray applications of ammonium 2,4-dichlorophenoxyacetate. Soybeans were also treated with soil applications of 2,4-dichlorophenoxyacetic acid.

2. Cabbage at three stages of growth (six to eight leaves, beginning to head, and heads 4 inches in diameter) were sprayed with this growth-regulator at rates of 0.3 or 3.0 pounds per acre. The two younger stages were killed by the compound, but the oldest plants were little inhibited in growth.

3. Soybeans at four stages of development were sprayed with the herbicide at a rate of 0.5 pounds per acre. The two younger stages (3 inches and 6–8 inches in height) were for a time severely retarded in growth and distorted by the treatment, but these plants eventually recovered and produced a normal yield of beans. The two older stages (early flowering and early pod) showed less visible response immediately following treatment, but subsequent yields of beans were significantly reduced. These data indicate that developing floral and ovarial structures are as severely affected by the herbicide as is young vegetative growth.

4. Soybeans at planting time and at three developmental stages also received soil applications of 2,4-dichlorophenoxyacetic acid at rates of 3, 10, or 20 pounds per acre. Only when the herbicide was applied at planting time was there a significant reduction in the yield of beans obtained. The lowest application rate caused no reduction in yield. Established plants were relatively unaffected.

5. Tomatoes at three developmental

stages received spray treatments of the herbicide at rates of 0.1 or 1.0 pounds per acre. Young plants (8–12 inches high), unless killed, showed a considerable capacity for vegetative recovery, with only a slightly reduced fruit yield. When plants were sprayed at the early flowering stage, no fruit was set. When treatment was made at the early fruiting stage, there was a reduction in yield, and the fruits were somewhat misshapen and frequently seedless.

6. Sweet potatoes and sugar beets were treated to determine the effect of the herbicidal spray on yield of root crops. No yield data were obtained for

sugar beets because treatment with 0.5 pound per acre killed plants at the two stages of development studied (5–7 inches in height and 12–14 inches in height, with roots 4–6 inches in length and thumb-size in diameter). Sweet potatoes at early runner stage or when vines completely covered the ground were treated at rates of 0.25 or 1.0 pounds per acre. Plants at the younger stage were killed by both rates of the compound and root yield of older plants was greatly inhibited, even at the lower treatment level. The susceptibility of the sweet potato to the growth-regulator decreased with increasing age and size of plant.

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EFFECTS OF CERTAIN GROWTH–REGULATING COMPOUNDS ON IRISH POTATOES[1]

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Introduction

Herbicidal agents selective in character might find widespread use in agriculture if they removed undesirable weeds without causing concurrent injury to a growing crop. It has been demonstrated that some of the substituted phenoxyacetic acids may be so employed in the control of broadleaf weeds in cereal corps (2). A herbicide which kills or

[1] Studies conducted at Camp Detrick, Frederick, Md., from January, 1945, to September, 1945, under the supervision of Dr. A. G. Norman.

inhibits plant growth indiscriminately, however, would be of little use in eliminating weeds from economic crops, since the crop would suffer as well.

During an extensive study of the effects of herbicidal agents on many broadleaf crops, it was noticed that, while 2,4,5-trichlorophenoxyacetic acid had a deleterious effect on the Irish potato, such agents as 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid produced only little inhibition of either vegetative growth or tuber yield.

Further studies were then carried out, and it is the purpose of this paper to present the results obtained.

EXPERIMENT I

This experiment was conducted to test the effect upon the potato of several plant growth-regulating substances containing the phenoxyacetic grouping and certain others unrelated in structure. The compounds were applied in aqueous sprays to potted greenhouse Irish potato plants about 1 foot in height. Each compound was applied in quadruplicate at the rate of 0.1 gm. per square yard in 50 ml. of aqueous solution. The applications were made in a spray chamber 0.5 square yard in area and 4 feet deep, with high-pressure Precision sprayers of 200-ml. capacity.

One month after treatment, plant height measurements were made, and 2 months after treatment, fresh weights of all tubers produced per half-gallon glazed pot were taken.

It is evident from the data of table 1 that ammonium 2,4,5-trichlorophenoxy-acetate significantly reduced the yield of tubers and also stunted the vegetative growth. One month after spray applications, the plants treated with this compound were stunted in growth and showed swelling of the pulvini and thickening of the stem immediately adjacent to the petiole. Potato plants sprayed with ammonium 2,4-dichlorophenoxy-acetate and 2-bromo-3-nitro-benzoic acid developed epinastic responses soon after treatment, but the plants appeared to have completely recovered within a few days.

The tubers from the plants treated with ammonium 2,4,5,-trichlorophenoxy-acetate were markedly reduced in size and had a warty, scab-like appearance (fig. 1), whereas none of the other compounds tested in this experiment caused evident injury to the tubers. Under the conditions of the experiment, ammonium 2-methyl-4-chlorophenoxyacetate reduced the yield of tubers significantly, but the tubers were normal in appearance.

It was noticed at the time of harvest that those plants that had been treated

TABLE 1

EFFECT OF CERTAIN GROWTH-REGULATORS ON IRISH POTATOES. APPLICATIONS IN AQUEOUS SPRAYS AT RATE OF 0.1 GM. PER SQUARE YARD IN 50 ML. OF SOLUTION. FIGURES REPRESENT MEAN FRESH WEIGHT OF TUBERS IN GRAMS AND MEAN HEIGHT OF PLANTS IN INCHES*

Treatment	Mean fresh-weight tubers[†]	Mean plant height[‡]
Untreated....................	64.4	31.6
NH$_4$ 2,4,5-trichlorophenoxyacetate..	9.6	16.2
NH$_4$ 2-methyl-4-chlorophenoxyacetate......................	45.0	29.5
NH$_4$ 2,4-dichlorophenoxyacetate....	56.7	26.5
NH$_4$ para-chlorophenoxyacetate....	62.6	26.6
NH$_4$ 2-bromo-3-nitrobenzoate......	57.6	26.2
NH$_4$ 2,3,5-triiodobenzoate.........	70.3	27.8
NH$_4$ 2-methyl-4,6-dichlorophenoxy-acetate......................	66.9	28.5
Isopropyl phenylcarbamate........	69.1	27.6
2,5-Dichlorophenoxyacetic acid.....	58.0	29.2
2,4-Dibromophenoxyacetic acid.....	61.8	28.4

* Minimum difference for significance between means at the 5% level of probability is 11.2 gm. (tubers) and 12.0 inches (height).

† Taken 2 months after treatment.

‡ Taken 1 month after treatment.

with 2,4-dichlorophenoxyacetic acid had set a considerable number of seed balls. None of the control plants or those treated with other compounds set any fruit. Although no attempt was made to test the seed for viability (they were harvested before maturity), it appears that this compound might be used to cause the setting of fruits after fertilization on such crops as potatoes, where premature abscission often limits or prevents the maturation of fruit.

EXPERIMENT 2

In Experiment 1 the greater effectiveness of ammonium 2,4,5-trichlorophenoxyacetate over ammonium 2,4-dichlorophenoxyacetate in causing inhibition of vegetative growth and tuber yield suggested that the chlorinated 5 position in the benzene ring might be responsible for the degree of specificity exhibited by this compound on the Irish potato. This experiment was designed to test this hypothesis, using, among others, some compounds with the 3 or 5 position chlorinated.

Greenhouse-grown Irish potato plants in 6-inch clay pots were arranged into treatment groups of four plants each when the plants were 3–8 inches in height. Care was exercised to have good uniformity between treatment groups, but there was considerable variation in size within treatment groups. Four plants were harvested at the time of treatment, and no tubers had formed at that time. Many of the compounds were difficultly water-soluble and accordingly were incorporated into oil, using tributylphosphate as a co-solvent (1). A low-volume spray of 5 ml. per square yard was applied, with each treatment solution containing 4 per cent tributylphosphate and 100 mg. of the compound. This treatment is roughly equivalent to a rate of 1 pound of acid per acre. The plants were sprayed in quadruplicate in a spray chamber 0.5 square yard in area and 4 feet deep, with a De Vilbiss atomizer operated under approximately 15 pounds air pressure. Fresh weights were taken of the tubers and of the vegetative

FIG. 1.—Typical injury to potato tubers caused by 2,4,5-trichlorophenoxyacetic acid applied by spray either to vegetative portions of the plant or to the soil. Injury is largely superficial.

portion of the plants 54 days after treatment.

Only the compounds which possessed the 2,4,5-chlorophenoxy configuration—2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetylchloride—caused significant decreases in the yield of potato tubers (table 2). One compound—3,5-dichlorophenoxyacetic acid—reduced the weight of vegetative growth but did not reduce the weight of tubers.

The significance of the 2,4,5-trichlorophenoxy configuration in causing injury to the potato was further borne out in another greenhouse comparison not re-

ported here, in which twenty substituted phenoxyacetic acids were tested on potatoes. Only the compounds possessing the 2,4,5-trichlorophenoxy configuration—namely, 2,4,5-trichlorophenoxyacetic anilide, 2,4,5-trichlorophenoxyacetic ortho-nitroanilide, 2,4,5-trichlorophenoxyacetic meta-nitroanilide, and 2,4,5-trichlorophenoxyacetonitrite—caused marked stunting of potato plants and injury to tubers. The injury was similar to that produced by 2,4,5-trichlorophenoxyacetic acid. No yield data were taken because of the large plant variability resulting from high summer temperatures.

It is evident, however, from the data of table 2 that little significance can be attributed to the chlorinated 5 position of the benzene ring in so far as phytocidal action on the potato is concerned.

EXPERIMENT 3

Extensive testing of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid has shown that they produce inhibition and death of many broadleaf plants if applied at relatively low rates. In the present study, emphasis was directed toward the actual response of field-grown potatoes to treatment with these compounds. Both aqueous- and oil-spray solutions of the compounds were applied, since comprehensive greenhouse studies indicated that the response of plants to growth-regulators might differ when the latter were applied in oil sprays instead of in aqueous sprays.

Irish Cobbler potatoes were planted April 9, 1945, in Frankstown silt loam in rows 3 feet apart, with cut "seed" pieces spaced at 12-inch intervals. Prior to planting, an application of 1000 pounds per acre of a 4-10-5 fertilizer was broadcast and disked into the soil. When the

plants were in the early bud stage (8–10 inches in height), aqueous and tributylphosphate-oil sprays of the three compounds were applied in quadruplicate at the rate of 0.1 gm. per square yard (about 1 pound per acre) in a spray volume of 10 ml. per square yard. The aqueous sprays were prepared by dissolving 8.0 gm. of each of the acids in

TABLE 2

EFFECT OF CERTAIN GROWTH-REGULATORS ON IRISH POTATOES. APPLICATION IN OIL SPRAYS AT RATE OF 0.1 GM. PER SQUARE YARD IN 5 ML. OF SOLUTION. FIGURES REPRESENT MEAN FRESH WEIGHT IN GRAMS TAKEN 54 DAYS AFTER TREATMENT*

Treatment	Mean fresh-weight tubers	Mean plant weight
Control (untreated)	35.4	57.9
4% tributylphosphate-oil control. . .	37.0	59.9
2,4,5-trichlorophenoxyacetic acid. . .	5.4†	28.2†
2,5-dimethylphenoxyacetic acid.	40.6	55.4
3-chlorophenoxyacetic acid.	38.2	59.4
2,5-dichlorophenoxyacetic acid.	21.8	64.6
3,5-dichlorophenoxyacetic acid.	50.2	43.0†
3,4-dichlorophenoxyacetic acid.	18.5	58.5
2,4,5-trichlorophenoxyacetyl chloride	13.2‡	36.1†
2,3,5-trichlorobenzoic acid.	29.1	63.5
Butyl 2,4-dichlorophenoxyacetate. . .	45.7	55.7

* Minimum difference for significance between means at 5% level of probabiliiy is 22.8 gm. (tubers) and 5.7 (vegetative plant).

† Significantly lower in yield than untreated at 99:1 odds.

‡ Significantly lower in yield than untreated at 19:1 odds.

800 ml. of water, to which had been added 5 ml. of NH_4OH and 3 ml. of diethylamine. The oil sprays were prepared by dissolving 8.0 gm. of each of the acids in 16 ml. of tributylphosphate and diluting to 800 ml. with no. 2 fuel oil. Each spray solution was applied uniformly to plots 12 × 12 feet with a modified De Vilbiss type CV paint-spray apparatus with 10 pounds constant air pressure from a pressure cylinder. The plots were protected from the effect of wind during the spray operation with canvas-covered

shields 4 feet high. The plants were harvested, and tubers were weighed from each plot on July 27 after all vines were dead.

TABLE 3

EFFECT OF THREE GROWTH-REGULATORS ON IRISH POTATOES. APPLICATIONS IN AQUEOUS AND OIL SPRAYS AT RATE OF 0.1 GM. PER SQUARE YARD IN 10 ML. OF SOLUTION. FIGURES ARE MEAN YIELDS PER PLOT (16 SQUARE YARDS) OF ALL TUBERS 57 DAYS AFTER SPRAY APPLICATION*

| TREATMENT | MEAN YIELD PER PLOT (IN POUNDS) | |
	Aqueous spray	Oil spray
2,4-dichlorophenoxyacetic acid.....	10.8	5.0
2-methyl 4-chlorophenoxyacetic acid	15.2	7.6
2,4,5-trichlorophenoxyacetic acid...	14.2	2.7
Control (untreated)...............	14.8	

* Minimum difference for significance between means at the 5% level of probability is 1.9 pounds.

It is evident from the data of table 3 that the oil sprays of the compounds caused a much greater reduction in yield of tubers than did the aqueous sprays. Although 2,4,5-trichlorophenoxyacetic acid did not reduce the yield of tubers substantially when applied in an aqueous spray, the quality of tubers produced was very poor (fig. 2). On the other hand, 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid applied to potato plants in either aqueous or oil sprays did not affect the quality of the tubers, but both reduced the yield of tubers significantly when applied in the oil spray.

EXPERIMENT 4

In view of the apparent specific action of 2,4,5-trichlorophenoxyacetic acid on the Irish potato when applied to the aerial portion of the plant, a preliminary study was made to determine whether the compound acted similarly when applied as a soil contaminant. Uniform
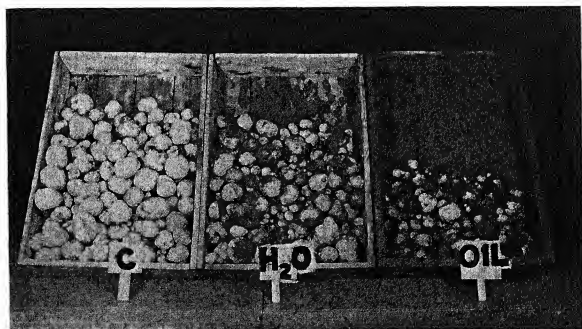
FIG. 2.—Effect upon potato tubers of 2,4,5-trichlorophenoxyacetic acid when applied to field-grown potatoes in water and oil sprays. The compound was applied at an early bud stage at the rate of 1 pound per acre in a volume of spray of 10 ml. per square yard. Greater reduction in yield and corky epidermal injury with oil spray.

potato plants, grown in 6-inch clay pots, were treated in duplicate when about 16–18 inches in height. The compounds 2,4-dichlorophenoxyacetic acid, ammonium 2,4,5-trichlorophenoxyacetate, and a 1:1 mixture of the two compounds were applied to the soil in 100 ml. of water at rates of 8, 24, and 40 mg. per pot, or approximately 4, 12, and 20 pounds per acre, respectively. Care was exercised to prevent the solutions from coming in direct contact with vegetative portions of the plants. All pots were uniformly surface-watered throughout the course of the experiment, and care was exercised to prevent leaching. Fresh weights were taken of the tubers 42 days after treatment.

Five hours after application all the treated plants showed bending of the stems and epinasty of the petioles. The degree of bending was positively correlated with the rate of application. Two days after treatment there was practically complete recovery from the effects produced by the 8 mg. per pot application of 2,4-dichlorophenoxyacetic acid, but plants in all other treatments retained some stem and petiole curvature.

One week after treatment, swelling of the pulvini was evident on all plants receiving ammonium 2,4,5-trichlorophenoxyacetate, either alone or in combination with 2,4-dichlorophenoxyacetic acid. A week later the leaves of many plants, which had showed swelling of the pulvini earlier, were broken off at the base of the petiole because of the brittle condition of the latter, probably resulting from the formation of callus-type growth with concurrent weakening of mechanical tissues. None of the rates of treatment with 2,4-dichlorophenoxyacetic acid caused apparent injury to the aerial portion of the plants 3 weeks after treatment.

A low yield (table 4) of tubers was produced by the plants treated with ammonium 2,4,5-trichlorophenoxyacetate or a 1:1 mixture of that compound and 2,4-dichlorophenoxyacetic acid, and the tubers were warty and small. Figure 1 shows typical injury. The 2,4-dichlorophenoxyacetic acid did not produce any apparent injury to the tubers.

Discussion

It is apparent from the experimental data presented that 2,4,5-trichlorophe-

TABLE 4

EFFECT UPON IRISH POTATOES OF GROWTH-REGU-
LATORS APPLIED TO SOIL. APPLICATIONS TO
SOIL IN 100-ML. AQUEOUS SOLUTION PER 6-
INCH CLAY POT. FIGURES REPRESENT PER
CENT OF TUBER YIELD PRODUCED BY UN-
TREATED PLANTS. YIELDS TAKEN 42 DAYS
AFTER TREATMENT. TWO PLANTS PER TREAT-
MENT

COMPOUND APPLIED	TREATMENT RATE PER POT		
	8 mg.	24 mg.	40 mg.
Untreated................	100.0	100.0	100.0
2,4-dichlorophenoxyacetic acid...................	104.8	83.0	71.0
2,4-dichlorophenoxyacetic acid and NH₄ 2,4,5-trichlorophenoxyacetate*.......	73.1	62.1	0.0
NH₄ 2,4,5-trichlorophenoxyacetate................	52.1	22.3	0.6

* A 1:1 mixture of these compounds was applied.

noxyacetic acid is extremely injurious to Irish potato tubers even at low rates and volumes of application in either oil or aqueous solution. Oil solutions of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid are likewise injurious, in that they cause some reduction in tuber yield, although, unlike 2,4,5-trichlorophenoxyacetic acid, they do not affect the marketable or edible quality of the tubers. Aqueous solutions

of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid, however, have no effect on either top growth or tuber yield when applied at rates and volumes of application which would be quite effective in inhibiting or killing the usual broadleaf weeds. None of the agents tested, in either oil or aqueous solution, had any marked effect on grasses.

The selective resistance of the Irish potato to 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid and the effectiveness of these chemicals in controlling the usual broadleaf weeds suggest that an aqueous herbicidal spray of either of these two agents, perhaps applied in conjunction with a fungicide or insecticide, can be effectively used as a weed-control measure.

Summary

1. The substance 2,4,5-trichlorophenoxyacetic acid and other compounds with the 2,4,5-trichlorophenoxy- configuration have been shown to have a decided herbicidal action on the Irish potato, while other phenoxyacetic acid derivatives applied in sprays at comparable rates produce markedly less and different effects.

2 The compound 2,4,5-trichlorophenoxyacetic acid, when applied to vegetative portions of Irish potato plants in aqueous or oil sprays, or to the soil, causes pronounced stunting and distortion of vegetative growth, swelling of the pulvini, and marked reduction in yield and quality of tubers.

3. The chlorinated 5 position of substituted phenoxyacetic acids does not appear to have any significance as far as the phytocidal action of such compounds on the potato is concerned, whereas the 2,4,5-trichloro- configuration appears to be significant.

4. The use of substituted phenoxyacetic acids, such as 2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid, may be of value for the selective control of weeds in potato fields. Further testing of such compounds for herbicidal use against weeds in potato fields is suggested.

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SOME EFFECTS OF PLANT GROWTH-REGULATORS ON SEED GERMINATION AND SEEDLING DEVELOPMENT[1]

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Introduction

Several recent reports in the literature have established that certain halogenated phenoxyacetic acid compounds and isopropyl-N-phenylcarbamate have herbicidal properties upon established plants (1, 2, 3, 4). The investigations reported in this paper were undertaken to determine the effects of these compounds upon the germination and early seedling stages of plants.

Experimentation and results

STUDIES IN MOIST CHAMBERS

Preliminary studies of the effect of 2,4-dichlorophenoxyacetic acid upon germination and seedling development of twenty-two cereal and broadleaf crops were made in glass chambers about 10 inches in diameter. Twenty-five seeds were placed upon four layers of filter paper, 100 ml. of solutions, which varied in concentration from 0.01 to 100 parts per million, were added, and the glass dish was placed in a darkroom maintained at $25°–27°$ C. Observations were made daily for 4 days. Tests were made with kidney beans, navy beans, soybeans, peas, cowpeas, Korean lespedeza, turnips, radishes, rutabagas, sugar beets, sunflowers, cotton, muskmelons, cucumbers, buckwheat, maize, wheat, oats, rice, sorghum, barley, and millet.

The general effect of the 2,4-dichlorophenoxyacetic acid to retard the rate of germination and to decrease the number of seeds which germinated. With

[1] Studies conducted at Camp Detrick, Frederick, Md., from February, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

dicotyledonous species, concentrations of 1.0 p.p.m. or greater caused swelling of both the hypocotyls and the roots, particularly in the region of the root-hypocotyl junction. Longitudinal ruptures of the surface frequently occurred in that region. The formation of lateral roots of dicotyledonous plants was inhibited completely by concentrations of 1.0 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. The response of cereals was somewhat different. The most striking symptoms were curvatures of the coleoptile and amorphous gall-like growths on the roots, particularly at the root tip. The plumules did not emerge from the coleoptile when cereals were treated with 1.0 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. Dissection of the coleoptiles showed that no development of the plumule had occurred.

No threshold concentration above which germination did not occur was apparent. Instead, the delay in the initiation of growth increased proportionally with increasing concentrations of 2,4-dichlorophenoxyacetic acid. Similarly, the number of abnormal seedlings and the severity of the symptoms increased gradually as the concentration was increased. As a result, no clear-cut distinction was possible between seeds which germinated and those which did not germinate, so that values for percentage of germination could not be assigned.

Quantitative measures of the inhibition of roots and shoots of spring wheat and cowpeas, given in table 1, were typical for the twenty-two cereal and broadleaf species treated with 2,4-di-

chlorophenoxyacetic acid. These data confirm the observation that inhibition was in direct proportion to the concentration. They also show that the roots of wheat and cowpeas were more sensitive than

TABLE 1

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON YOUNG SEEDLINGS OF SPRING WHEAT AND COWPEAS GROWN IN MOIST CHAMBERS*

CONCENTRATION OF 2,4-DICHLORO-PHENOXYACETIC ACID (P.P.M.)	WHEAT		COWPEAS	
	Length of root (mm.)	Length of shoot (mm.)	Length of root (mm.)	Length of shoot (mm.)
0.	60.7	44.6	47.7	29.4
0.01.	48.0	42.8	40.0	23.6
0.1.	48.7	43.3	29.4	22.4
1.0.	20.2	35.0	12.7	13.7
10.0.	10.0	22.1	3.0	9.4
100.0.	1.0	3.4	1.6	8.2

* Mean of four or more dishes, each containing approximately twenty-five seeds.

TABLE 2

EFFECT OF APPLICATIONS OF SOLUTIONS OF 10 P.P.M. OF SEVERAL GROWTH-REGULATORS UPON THE GROWTH OF ROOTS OF MAIZE GROWN IN MOIST CHAMBERS*

Compound	Root length (mm.)
4-chlorophenoxyacetic acid.	42
2,4-dichlorophenoxyacetic acid.	19
2-methyl-4-chlorophenoxyacetic acid	19
2,4,5-trichlorophenoxyacetic acid. . . .	20
Isopropyl-N-phenylcarbamate.	78
Control.	129

* Mean of four dishes, each containing approximately twenty-five seeds.

the shoots. This was likewise true in all other species tested. The germination and seedling development of a few species, particularly sorghum, peas, and navy beans, deviated from the usual response in that concentrations of 0.01 and 0.1 p.p.m. appeared to be stimulating.

A comparison of the effect of 2,4-dichlorophenoxyacetic acid with three other chlorophenoxyacetic acids and isopropyl-N-phenylcarbamate upon the germination of maize is given in table 2. The symptoms produced by 4-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid were the same as those caused by 2,4-dichlorophenoxyacetic acid. However, 4-chlorophenoxyacetic acid was less effective than the other compounds. Isopropyl-N-phenyl carbamate did not cause curvatures or gall-like growths, nor did it inhibit growth as much as the chlorophenoxyacetic acids.

STUDIES IN SOIL

The results in moist chambers were compared with soil applications by preparing solutions which contained 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 mg. of 2,4-dichlorophenoxyacetic acid per 50 ml. of 0.5 per cent Carbowax solution and applying these solutions to pots which contained about 1 pound of soil. After 3 days, the soil was removed, thoroughly mixed, replaced in the pots, and seeds of kidney beans, wheat, or oats were planted. Nine days after planting, when kidney-bean plants in the untreated pots were about 5 inches tall and wheat and oat plants 1–2 inches tall, all seedlings were removed from the soil and examined. At that time no emergence had occurred in pots treated with 6.25 mg. or more of 2,4-dichlorophenoxyacetic acid.

KIDNEY BEANS.—The effect of 2,4-dichlorophenoxyacetic acid upon kidney beans is shown in figure 1. When 3.12 mg. or more of the growth-regulator were applied, normal roots were severely inhibited; but 1.56 and 0.78 mg. appeared to stimulate growth of roots. However,

the shoots were stunted by even the lowest rate of application.

Histological studies of some of these plants, with methods described previously (2), showed that 2,4-dichlorophenoxyacetic acid had caused abnormalities to develop in the anatomy of the seedlings. In the control plants the hypocotyl was hollow as a result of the splitting of the pith. The strands of primary xylem and phloem were clearly developed and discontinuous; and differentiation of the cambium had occurred, although no secondary tissues were as yet laid down (fig. 2). With applications of 2,4-dichlorophenoxyacetic acid at rates of 1.56 and 3.13 mg. per pound of soil, a noticeable shortening of the hypocotyl was evident. Histologically, four differences were noted in transverse sections (fig. 3). These were (1) slight delay in the maturation of the primary conductive tissues, (2) failure of the pith to split to give rise to a hollow stem, (3) enlargement of the cortical and pith cells, and (4) slight stimulation of cell proliferation in regions of the primary phloem and the rays. The cambium was not clearly defined, but an area of actively dividing cells between the primary xylem and the phloem in the vascular bundles might have been interpreted as cambial in nature. Inability to distinguish between the inner cortical cells and the endodermis made observations in this region difficult and uncertain, but scattered proliferation had occurred. Where recognizable, the pericycle seemed unaffected by the 2,4-dichlorophenoxyacetic acid.

With higher rates of application of 2,4-dichlorophenoxyacetic acid, especially when more than 12.5 mg. per pound of soil were applied, there was increased stunting and distortion of the hypocotyl

FIG. 1.—Kidney beans germinated in soil treated with 2,4-dichlorophenoxyacetic acid. C, control; 1, 25 mg.; 2, 12.5 mg.; 3, 6.25 mg.; 4, 3.12 mg.; 5, 1.56 mg.; and 6, 0.78 mg. per pot.

region, with marked hypertrophy of the adventitious root system. The pith was solid, and there was considerable inhibition of the differentiation of conductive tissue and pericyclic fibers. Most striking of all was the development of fasciated roots, which generally possessed a tetrarch arrangement.

FIGS. 2–4.—Transverse sections of the hypocotyl of kidney bean. Fig. 2, untreated. Fig. 3, treated with 1.56 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil. Solid pith and increased diameter caused by cell proliferation and enlargement. Fig. 4, treated with 50 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil. Solid pith, root primordia, and disrupted cortex.

The development of the primary root was nearly completely inhibited. Figure 4 shows a transverse section through the hypocotyl after treatment with 2,4-dichlorophenoxyacetic acid at 50 mg. per pound of soil. The larger adventitious roots arose from, and over, the region of the medullary rays; but the primary phloem, and possibly the cambium, appeared to contribute cellular elements as well as the rays. Between the larger roots there extended broad proliferated areas, which generally consisted of cells derived from both ray and phloem elements and which sometimes gave rise to other smaller root primordia. These, as well as the apices of the larger roots, consisted of many small, deeply staining cells, which appeared to have undergone very rapid cell proliferation. No vascular differentiation occurred in the roots other than the formation of a few scattered and disorganized tracheid-like cells and some elongation of the cells making up the central core. The cortex was disrupted by the outward development of root primordia (fig. 4).

WHEAT AND OATS.—The effect of the 2,4-dichlorophenoxyacetic acid upon wheat and oats was to cause curvature and stunting of the coleoptiles (figs. 5 and 6) and severe inhibition of the roots. The development of the roots was more affected than that of the coleoptiles. Although an application of 0.78 mg. had little or no effect upon the coleoptiles, it caused severe abnormalities in root development (figs. 5 and 6). Applications of 6.25 mg. or more prevented all development of the roots.

Further tests of the effect upon germination of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyaceticacid, and isopropyl-N-phenylcarbamate were made upon soybeans, turnips, buckwheat, and barley, grown in standard greenhouse flats. The compounds were applied to the soil in 100 ml. of aqueous solution in amounts to give the equivalent of applications of 2 or 5 pounds per acre. Three days later the soil was removed, thoroughly mixed, replaced in the flats, and the seeds planted.

Six days after planting, normal emergence had occurred in all control flats. No

FIG. 5.—Oat seedlings germinated in soil treated with 2,4-dichlorophenoxyacetic acid. *1*, control; *2*, 50 mg.; *3*, 25 mg.; *4*, 12.5 mg.; *5*, 6.25 mg.; *6*, 3.13 mg.; *7*, 1.56 mg.; and *8*, 0.78 mg. per pound of soil.

FIG. 6.—Wheat seedlings germinated in soil treated with 2,4-dichlorophenoxyacetic acid. *1*, control. *2*, 50 mg.; *3*, 25 mg.; *4*, 12.5 mg.; *5*, 6.25 mg.; *6*, 3.13 mg.; *7*, 1.56 mg.; and *8*, 0.78 mg. per pound of soil.

emergence of turnips or soybeans had occurred where 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid had been applied; and, although some buckwheat and barley plants had emerged, the plants were stunted, particularly at the 5-pound rate of application. Isopropyl-N-phenylcarbamate had no noticeable affect upon the turnips or soybeans but had prevented emergence of any buckwheat or barley plants.

growths. Buckwheat, a dicotyledonous plant, thus behaves like cereals in its response to chlorophenoxyacetic acids and isopropyl-N-phenylcarbamate.

When the selective herbicidal properties of 2,4-dichlorophenoxyacetic acid and other phenoxyacetic acids for broadleaf species and isopropyl-N-phenylcarbamate for grass species became apparent, trials were initiated to test for any general herbicidal properties of mixtures

TABLE 3

GERMINATION OF THREE DICOTYLEDONOUS AND ONE CEREAL SPECIES TREATED WITH TWO CHLOROPHENOXYACETIC ACIDS AND ISOPROPYL-N-PHENY-CARBAMATE, APPLIED TO THE SOIL IN AQUEOUS SOLUTIONS

COMPOUND	EQUIVA-LENT RATE PER ACRE (POUNDS)	NUMBER OF PLANTS*			
		Soybean	Buck-wheat	Turnip	Barley
Control................................	....	11	22	41	14
2,4-dichlorophenoxyacetic acid............	2	5	19	0	13
2-methyl-4-chlorophenoxyacetic acid.......	2	1	19	0	14
Isopropyl-N-phenylcarbamate............	2	12	0	38	0
Control................................	....	13	20	39	15
2,4-dichlorophenoxyacetic acid............	5	0	14	0	8
2-methyl-4-chlorophenoxyacetic acid.......	5	0	13	0	12
Isopropyl-N-phenylcarbamate............	5	16	0	38	0

* Mean of four standard greenhouse flats.

Sixteen days after germination, a few badly stunted turnip and soybean plants had emerged from flats treated with 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid, but the emergence of buckwheat and barley was nearly complete, though the plants were stunted.

The emergence 24 days after planting, given in table 3, shows the selectivity of the compounds. Although the buckwheat and the barley emerged nearly completely, the entire plants were stunted by the chlorophenoxyacetic acids, particularly the roots, which were short, swollen, and frequently covered by gall-like out-

of the two types of compounds. Because it was also considered possible that the compounds might complement the action of one another, the experiments were designed so that interactionary effects, if present, could be determined statistically. The treatments applied were: (A) 2,4-dichlorophenoxyacetic acid, (B) 2,4,5,-trichlorophenoxyacetic acid, and (C) isopropyl-N-phenylcarbamate, all at 3 mg. per pot; mixtures of $A \times B$, $A \times C$, $B \times C$, all at a rate of $1\frac{1}{2}$ mg. of each compound per pot; and a mixture of $A \times B \times C$ at 1 mg. of each compound per pot. All treatments were applied to the soil in 25 ml. of water.

Oats were used as a representative of the grasses, and soybeans for the broadleaf species. Three series of tests were conducted. In the first series, twenty-five oat or 6 soybean seeds were planted per pot, and four pots of each species were given each treatment immediately after seeding. In the second and third series

growth from each pot was used as a replicate in the analysis of the data.

It is readily seen from table 4 that 2, 4-dichlorophenoxyacetic acid and 2,4,5,-trichlorophenoxyacetic acid greatly decreased the growth of soybeans but did not cause significant reduction in the growth of oats; the converse was true for

TABLE 4

FRESH VEGETATIVE WEIGHT (IN GM.) OF OATS AND SOYBEANS TREATED WITH THREE
GROWTH-REGULATORS AND MIXTURES OF THE GROWTH-REGULATORS APPLIED
IN AQUEOUS SOLUTION TO THE SOIL

COMPOUND	RATE IN MG. PER POT	STAGE AT TIME OF TREATMENT					
		Oats*		Soybeans*		Oats†	Soybeans†
		Seeding	Estab-lished	Seeding	Estab-lished	Estab-lished	Estab-lished
Control.	0	11.55	8.56	12.72	12.32	1.60	1.87
2,4-dichlorophenoxyacetic acid. . .·. . . .	3	9.41	10.02	8.34‡	7.70‡	1.53	0.83‡
2,4,5-trichlorophenoxyacetic acid.	3	9.99	9.60	6.66‡	5.28‡	1.32§	0.80‡
Isopropyl-N-phenylcarbamate.	3	4.23‡	2.52‡	13.59	10.93	0.28‡	1.65
2,4-dichlorophenoxyacetic acid *plus* 2,4,5-trichlorophenoxyacetic acid. . .	1½+1½	10.68	7.68	10.46	6.63‡	0.21‡	0.41‡
2,4-dichlorophenoxyacetic acid *plus* Isopropyl-N-phenylcarbamate	1½+1½	8.10‡	2.18‡	10.22	12.64	0.28‡	0.96‡
2,4,5-trichlorophenoxyacetic acid *plus* isopropyl-N-phenylcarbamate.	1½+1½	4.67‡	1.99‡	9.87	8.55‡	0.29‡	0.98‡
2,4-dichlorophenoxyacetic acid *plus* 2,4,5-trichlorophenoxyacetic acid *plus* isopropyl-N-phenylcarbamate. .	1+1+1	7.99‡	3.44‡	10.49	7.25‡	0.23‡	0.23‡

* Mean of four pots.

† Mean of eight pots.

‡ Lower than the control at odds exceeding 1:19.

§ Lower than the control at odds exceeding 1:99.

an excess of seeds was planted, and the pots were thinned to fifteen oat plants or six soybean plants immediately before treatment, at which time the oats were 3 inches tall and the soybeans 2 inches tall. Four pots were given each treatment in series 2, and eight pots were given each treatment in series 3. The pots were subirrigated to avoid leaching. Twenty-three days after treatment the aerial portions of the plants were cut and weighed. The weight of fresh aerial

isopropyl-N-phenylcarbamate. The effects of the mixtures were not uniform in the three series of experiments; but, in general, mixtures of either of these chlorophenoxyacetic acids with isopropyl-N-phenylcarbamate were effective in decreasing the growth of both oats and soybeans (table 4).

Analysis of the interactionary effects showed that the greater portion, if not all, of the effect upon oats was caused by isopropyl-N-phenylcarbamate, that the

phenoxyacetic acids similarly caused the greater portion of the decrease in growth of the soybeans, and that any interactions of the compounds were unimportant. These results indicated that isopropyl-N-phenylcarbamate and 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acids are not complementary in action.

Discussion

In general, 2,4-diclorophenoxyacetic acid inhibited germination, decreased the growth of young seedlings, and caused abnormalities in the anatomy of the seedlings of twenty-two crop plants. More limited tests indicated that 4-chlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 2-methyl-4-chlorophenoxyacetic acid produced the same responses in germinating seeds.

In moist chambers the inhibition of growth of germinating cereal and dicotyledonous seeds was not greatly different over a wide range of concentrations of 2,4-dichlorophenoxyacetic acid. However, chlorophenoxyacetic acids applied to the soil nearly completely prevented the germination and establishment of broadleaf species at rates of application which merely stunted cereals. The behavior of isopropyl-N-phenylcarbamate was quite different, in that it had much less effect upon germinating cereal seeds in moist chambers than chlorophenoxyacetic acids, but it completely prevented the establishment of cereals in soil.

The foregoing results show that the specificity of certain growth-regulating compounds may be different when applied to germinating seeds and to established plants, and they may also indicate some influence of the soil upon the response obtained. Consequently, caution is necessary in evaluating the effectiveness of a weed-killer upon any single

species on the basis of germinating-seed tests in moist chambers or upon tests made at a single stage of development.

In the soil a mixture of either 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid with isopropyl-N-phenylcarbamate proved an effective herbicide against young seedlings of both broadleaf and cereal species. However, mixtures of these compounds were not more effective on soybeans and oats than the compounds alone, and evidence of clear-cut interactionary or complementary effects was not obtained.

The lack of specificity of 2,4-dichlorophenoxyacetic acid in inhibiting the germination of seeds of twenty-two crop plants, which indicated a more general herbicidal effect upon very young than upon older plants, may have practical application in weed-control programs.

Summary

1. In studies in moist chambers, 2,4-dichlorophenoxyacetic acid was found to delay germination and to cause abnormalities in the seedling development of twenty-two broadleaf and cereal species. Isopropyl-N-phenylcarbamate was less effective than four chlorophenoxyacetic acids in reducing growth of seedlings of maize.

2. 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid applied to the soil prevented the germination of broadleaf species at rates which stunted cereals but did not prevent establishment. Isopropyl-N-phenylcarbamate prevented the establishment of cereals at rates which had no effect upon broadleaf species. Buckwheat, a dicotyledonous species, responded like a cereal.

3. Mixtures of chlorophenoxyacetic acids with isopropyl-N-phenylcarba-

mate inhibited the development of seedlings of both broadleaf and cereal species and hence have promise as general herbicides, but they gave no evidence of complementary action of the two types of compounds.

4. The general lack of specificity of 2,4-dichlorophenoxyacetic acid in inhibiting the germination of seeds of twenty-two different species may have practical applications in weed-control programs.

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PERSISTENCE OF SOME PLANT GROWTH-REGULATORS WHEN APPLIED TO THE SOIL IN HERBICIDAL TREATMENTS[1]

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Introduction

The herbicidal action of the halogenated phenoxyacetic acids has been amply demonstrated. The possibility that some compounds of this series also possess a selectivity of action on certain weed species further emphasizes the importance of the phenoxyacetic acids in any weed-control program (3). Their judicious use, however, depends upon a knowledge of their action under many and varied conditions.

Since with such compounds it is possible to achieve herbicidal effects by treatment of the soil, as well as by application of aqueous or oil solutions to the plant itself, it is of importance to determine their behavior following incorporation into the soil. SLADE, TEMPLEMAN,

and SEXTON (2) demonstrated that 2-methyl-4-chlorophenoxyacetic acid, an excellent herbicidal compound, persisted for some time in the soil. NUTMAN, THORNTON, and QUASTEL (1), on the other hand, found that 2,4-dichlorophenoxyacetic acid, when applied to the soil in small amounts, lost its toxicity completely within 3–6 days. They also demonstrated that leaching equivalent to 1.4 inches of water resulted in a loss of activity that was just significant, but that far greater volumes of water did not completely remove this compound from the soil. Reduction in herbicidal effectiveness or total loss in activity of the herbicide can result from leaching, from hydrolysis or decomposition due to the action of the micro-organisms of the soil, or from inactivation due to adsorption or fixation by soil colloids.

The investigations reported below are

the results of studies undertaken to determine the persistence of several growth-regulators when added to the soil.

Experimentation and results

GREENHOUSE EXPERIMENTS

EXPERIMENT 1.—The purpose of this experiment was to determine whether 2,4-dichlorophenoxyacetic acid is readily leached from a greenhouse soil. A stock solution was prepared by dissolving 1 gm. of the chemical in 5 gm. of melted Carbowax 1500 and adding this immediately, drop by drop, to 1 liter of warm water. Solutions containing various amounts of 2,4-dichlorophenoxyacetic acid were prepared by dilution from the stock solution. Half-gallon glazed crocks, which had a glass-wool filter placed at the drainage outlet, were used for soil treatment. The drainage outlet was fitted with a one-hole rubber stopper, which had a small glass tube inserted in order to allow collection of the leachate in Erlenmeyer flasks. The crocks were filled with 2500 gm. of soil, consisting of a mixture of one part silt loam and one part medium sand. Solutions of 2,4-dichlorophenoxyacetic acid containing 100, 50, 12.6, 6.4, or 3.2 mg., respectively, were added to the crocks. After the soil had been treated with the growth substance or herbicide, enough additional water was applied to bring the soil to its predetermined water-holding capacity. It was assumed that in this way the 2,4-dichlorophenoxyacetic acid would be equally distributed throughout the soil mass. After treatment, the soil was allowed to stand 2 days before leaching.

Each series of crocks was then leached with an amount of water equivalent to 1.5, 3.0, or 4.5 inches of rain. The leachate was collected and tested for toxicity by placing one drop on young red kidney bean and tomato leaves at the junction of the petiole and the expanded leaf blade. The leached soils, after partial drying, were tested for residual 2,4-dichlorophenoxyacetic acid by observing the growth responses of young tomato plants transplanted into them.

Parts of the leachates from the soils which had been leached with water equivalent to 1.5, 3.0, or 4.5 inches of rain were combined and, after filtration, were allowed to evaporate slowly so that the growth substance could crystallize. It was found that white needle-like crystals were formed. These crystals were dissolved in boiling benzene, an equal amount of petroleum ether added, the solution cooled to o° C., and melting-point determinations were made on the recrystallized product. It was found in each case that the recrystallized compound had a melting-point of 139° C. (uncorrected), which is approximately the melting-point of 2,4-dichlorophenoxyacetic acid.

When one drop of an 0.5% Carbowax aqueous solution containing a small amount of the recrystallized compound was placed at the junction of the petiole and the expanded leaf blades of young tomato and bean plants, stem curvature and epinasty characteristic of plants treated with 2,4-dichlorophenoxyacetic acid were obtained within a few hours. Similar reactions occurred when the leachates themselves were tested for toxicity.

It was further found that when young tomato plants were transplanted into soils which had been treated initially with 100 mg. of the herbicide per pot and leached with 1.5 and 3.0 inches of water, respectively, plant responses characteristically caused by 2,4-dichlorophenoxyacetic acid developed (fig. 1). On the other hand, if the soil was leached

with 4.5 inches of water, there were no plant responses due to the residual growth substance. It was further found that when young tomato plants were transplanted into soils which had been treated with 3.2 mg. of 2,4-dichlorophenoxyacetic acid and leached with 1.5, 3.0, and 4.5 inches of water, respectively, normal leaves were produced (fig. 2).

It may therefore be concluded that the 2,4-dichlorophenoxyacetic acid was leached from the soil or that, if any of the growth-regulator remained in the soil, it was inactivated in some manner as yet undetermined.

EXPERIMENT 2.—Because of the nature of the information obtained in Experiment 1, it seemed pertinent to study
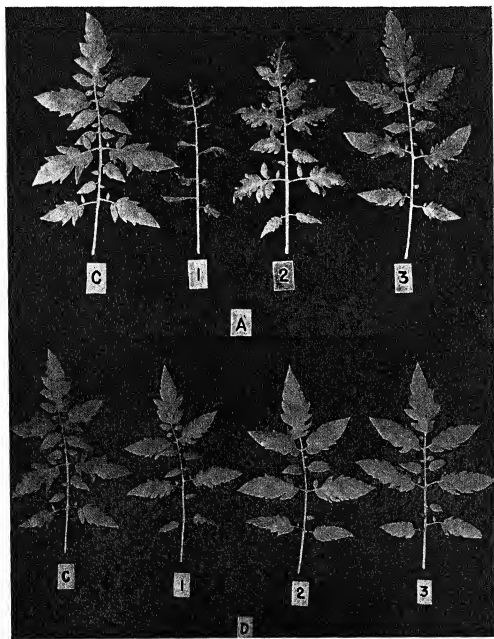
FIGS. 1, 2.—Fig. 1, Effects of residual 2,4-dichlorophenoxyacetic acid upon the leaves of young tomato plants. The soil had been treated with 100 mg. of compound per pot and leached with water equivalent to various amounts of rainfall prior to transplanting. C, control, 1–1.5, 2–3.0, and 3–4.5 inches of rainfall. Fig. 2, effects of residual 2,4-dichlorophenoxyacetic acid upon the leaves of young tomato plants. The soil had been treated with 3.2 mg. of chemical per pot and leached with water equivalent to various amounts of rainfall prior to transplanting. C, control, 1–1.5, 2–3.0, and 3–4.5 inches of rainfall.

the persistence of 2,4-dichlorophenoxy-acetic acid in soil which was not subject-ed to leaching. A greenhouse experiment which ran for 12 weeks was set up for this investigation. A stock solution was prepared by dissolving 390 mg. of puri-fied 2,4-dichlorophenoxyacetic acid in 650 ml. of 0.5% Carbowax aqueous solu-tion. Several series of half-gallon pots were filled with greenhouse soil (5 pounds to each pot), and the stock solution of 2,4-dichlorophenoxyacetic acid added in amounts necessary to give 1, 2, 3, 4, 5, 6, 8, 10, or 12 mg. per pound of soil. After the soil had been treated, 150 ml. of wa-ter were added to each crock in order to distribute the solution through the soil mass. The soil was not subjected to leach-ing but was kept just moist during the period that was allowed to elapse before planting.

Prior to planting, the soil was par-tially dried, removed, thoroughly mixed, and replaced in the pots to avoid the pos-sibility of an unequal distribution of 2,4-dichlorophenoxyacetic acid. At intervals of 4 weeks, red kidney-bean seeds were planted and one tomato plant, 10 days old, was transplanted into each pot. A new series of pots was started every 2 weeks, so that, by comparison of plant responses, information could be obtained as to the rate of decreasing effectiveness of the herbicide.

Initially, it was found that tomato plants were unable to establish them-selves in any of the treated pots. After the 8th week, however, even at the higher application rates of 4–12 mg. per pound of soil, normal plants developed. At 6 weeks, plants developed normally only if the original application was 3 mg. per pound of soil or less, and at 4 weeks only if the initial application did not exceed 1 mg. per pound of soil. The observations with respect to seed germination were

similar. After a period of 6 weeks, all seeds germinated, no matter how high the initial application had been. At 4 weeks, only about 50% germination was obtained if the initial application of 2,4-dichlorophenoxyacetic acid was 4 mg. per pound of soil or higher. It therefore appears that relatively heavy applica-tions of 2,4-dichlorophenoxyacetic acid are likely to become inactivated in soil in 6–8 weeks. These experiments, it is to be emphasized, were carried out under greenhouse conditions, with watering ad-justed so that no drainage losses from the pots occurred.

FIELD EXPERIMENTS

These experiments were carried out on a silt-loam soil with a pH of 6.5. The soil had been cultivated the previous year, and the field was prepared for crops ac-cording to the usual agronomic practices.

EXPERIMENT 1.—The object of this experiment was to compare the persist-ence of several growth substances when applied to a field soil. Plots (10 × 20 feet) were prepared and made approximately level. Around each plot a small dike was constructed to prevent surface washing. Lengthwise through the center of each plot a small dike was similarly construct-ed to divide it into a treated area and an untreated control area. In the design of the experiment an untreated control plot was placed adjacent to a treated plot.

Just prior to planting, the seed beds were carefully prepared and the growth substances applied at the excessively heavy rate of 50 pounds per acre. The growth substances tested were 2,4-di-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and isopropyl N-phenylcarbamate. Measured amounts of these substances were added to 200-gm. portions of Fuller's earth, and these mixtures were dusted on the soil. After

the applications had been made, the surface layer of soil was carefully raked.

Soybeans were used as the test crop. Sixty seeds were planted in rows 5 feet long and 14 inches apart. New plantings were made during the experiment at 17-day intervals after the initial planting.

The results of this investigation are summarized in figure 3. It is to be noted that 34 days after the initial application there was enough residual growth substance remaining in the soil to cause total inhibition of germinating soybeans. At 51 days, about 40% of the soybeans survived in the soil which had been treated with isopropyl N-phenylcarbamate; and there was no germination in the soil which had been treated with either 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid. On the other hand, 90 days after the soil had been treated with 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate there was more than 90% survival of soybean plants. There was, then, less than 25% survival of soybean plants in the soil which had been treated with 2-methyl-4-chlorophenoxyacetic acid. During the period of the investigation, several heavy rains occurred, which may have leached considerable amounts of the growth-regulators from the soil.

EXPERIMENT 2.—In this study a comparison of persistence was made between two growth substances on two different test crops. The soil was prepared as in experiment 1 and the same rate of application was used. The growth substances tested were 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate, and the crops were soybeans and oats.

It was found that, as measured by the germination and survival of oats and despite the excessively high rate of application of 50 pounds per acre, isopropyl-

phenylcarbamate did not persist in the soil for more than 60 days (table 1). On the other hand, as measured by residual toxicity toward soybeans, 2,4-dichloro-
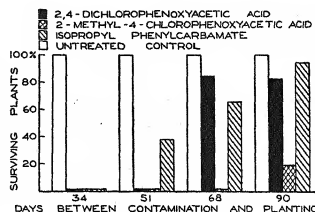
FIG. 3.—Persistence in the soil of three growth-regulating substances. The compounds were applied at the rate of 50 pounds per acre, and germination and survival of soybeans were used to indicate presence of compound in the soil.

TABLE 1

COMPARISON OF THE PERSISTENCE OF TWO GROWTH SUBSTANCES WHEN ADDED TO THE SOIL UNDER FIELD CONDITIONS AS MEASURED BY THE GERMINATION OF SOYBEANS (2,4-DICHLOROPHENOXYACETIC ACID) AND OATS (ISOPROPYL N-PHENYLCARBAMATE)

TREATMENT	PERCENTAGE OF GERMINATION AND SURVIVAL AFTER				
	10 days	26 days	40 days	60 days	80 days
2,4-dichlorophenoxyacetic acid	0	0	0	75	89
Control	96	94	98	100	100
Isopropylphenylcarbamate	0	20	45	95	98
Control	93	98	100	96	97

phenoxyacetic acid was more persistent in soil, though, after 80 days, the germination of soybeans was almost normal.

EXPERIMENT 3.—The purpose of this experiment was to observe the persistence of 2,4-dichlorophenoxyacetic acid

and 2,4,5-trichlorophenoxyacetic acid in soil which had produced a crop of potatoes.

The soil of a plot of Irish potatoes had been treated with these growth substances at the rates of 3, 10, and 20 pounds per acre. About 90 days after the potatoes had been planted and 10 days after harvesting, the field was carefully disked and replanted to soybeans. It was found that there were no residual effects from the 3-pound per acre application of either 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid. However, in the cases of 10- and 20-pound applications, 2,4,5-trichlorophenoxyacetic acid showed greater residual effects on soybeans than did 2,4-dichlorophenoxyacetic acid. Total inhibition of soybean germination was produced by 2,4,5-trichlorophenoxyacetic acid when applied originally at 20 pounds per acre, while 2,4-dichlorophenoxyacetic acid at the same rate did not produce a noticeable effect.

Discussion

The experiments described above revealed (1) that 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate, even when applied in excessive amounts, readily leached from the soil and that when leaching was prevented, the former compound was inactivated; and (2) that 2-methyl-4-chlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid are much more persistent, and were herbicidally active for a longer period of time than was 2,4-dichlorophenoxyacetic acid. The data tend to suggest, therefore, that, when using a herbicide as a soil contaminant, proper selection of the herbicide is important. The 2,4-dichlorophenoxyacetic acid is effective for a short time but rapidly loses its effectiveness through leaching or

inactivation. The 2-methyl-4-chlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, on the other hand, are equally effective in inhibiting growth and germination but differ in being effective over a greater period of time. At the present time no information is available to explain this difference in persistence.

Summary

1. Greenhouse experiments for determining the leachability and persistence of 2,4-dichlorophenoxyacetic acid in soil have been described.

2. When a soil was contaminated with 2,4-dichlorophenoxyacetic acid and then leached, it was found that the compound was present in the leachate.

3. It was demonstrated that normal plants developed in a greenhouse soil 8 weeks after the latter had been treated with high rates of 2,4-dichlorophenoxyacetic acid. Observations with respect to seed germination were similar. It may therefore be concluded that, under greenhouse conditions, this herbicide does not remain active in unleached soil for longer than 8 weeks.

4. In the field it was shown that 2,4-dichlorophenoxyacetic acid did not persist in soil for more than 80 days after treatment, while isopropyl N-phenylcarbamate had apparently disappeared within 60 days after treatment.

5. A field comparison was made of the persistence of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and isopropylphenylcarbamate applied at a heavy rate to the soil. It was found that after 68 days the soil still contained enough 2-methyl-4-chlorophenoxyacetic acid to be toxic to soybeans. On the other hand, 68 days after the soil had been treated with 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate, there was practically a complete

disappearance of the herbicides from the soil even when the compounds were applied at rates far above those likely to be used in practice.

6. 2,4,5-trichlorophenoxyacetic acid retains its herbicidal effectiveness in the soil for a longer period of time than does 2,4-dichlorophenoxyacetic acid.

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THE ACTION OF ISOPROPYLPHENYL-CARBAMATE UPON PLANTS[1]

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The physiological activity of compounds of the urethane series upon animals has been known for over thirty years (1). The first known reference to experimentation to determine the effect of these compounds upon plants is that of FRIESEN (2), who reported the retardation of seed germination and abnormal growth of oat and wheat seedlings following treatment with dilute solutions of phenylurethane. Since 1929, LEFÈVRE (4), SIMONET and GUINOCHET (5), GUINOCHET (3), SWINGLE and MAYER (6), and THOMPSON et al. (8) have reported the effects of various urethanes upon plants. TEMPLEMAN and SEXTON (7) were the first to apply isopropylphenylcarbamate, which they found to be more active upon cereals than some other urethanes but without effect upon certain dicotyledonous species.

This paper presents the results from several greenhouse and field experiments

conducted to compare the herbicidal properties of O-isopropyl N-phenylcarbamate with those of some of the more active phenoxyacetic acid derivatives.

Soil applications

EXPERIMENT I

Aqueous solutions of isopropylphenylcarbamate, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid were applied to oats and soybeans. Twenty-five oat seeds or six soybean seeds were planted per 4-inch pot, which contained approximately 1 pound of soil. One group of pots was treated immediately after seeding with 3 or 6 mg. of the growth regulator applied to the soil in 25 ml. of water. The other group of pots was not treated until the oat plants were about 4 inches, and the soybeans 3 inches, in height, with the first trifoliate leaf partially unfolded. The second group was thinned to fifteen oat plants and three soybean plants before treatment. Care was taken to apply the solution

only to the soil and not upon the seedling plants. The pots were placed in saucers and subirrigated to prevent leaching of the chemicals. In both groups, four pots were given each treatment, and the green weight of the aerial portions of the plants in a single pot was used as a replicate in the analysis of the data. The plants were cut and weighed 23 days after treatment.

In untreated pots, twenty-one to twenty-five oat seeds germinated and made good growth. In pots treated with

about 14 per cent of that of the plants in the untreated pots (table 1).

The same effect, but to a lesser degree, was observed when oats were treated at seeding with 3 mg. of isopropylphenylcarbamate. Usually three to five plants made some growth. The fresh weight of the plants at time of harvest was about one-third that of the untreated controls (table 1).

When isopropylphenylcarbamate was applied to established oat plants, no ef-

TABLE 1

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE, 2,4-DICHLOROPHENOXYACETIC ACID, AND
2,4,5-TRICHLOROPHENOXYACETIC ACID UPON THE VEGETATIVE WEIGHT (IN GRAMS) OF
OATS AND SOYBEANS, GROWN IN 4-INCH POTS, AND CUT 23 DAYS AFTER TREATMENT

TREATMENT	RATE (MG. PER POT)	STAGE AT TIME OF TREATMENT			
		Oats		Soybeans	
		Seeding	4 inches	Seeding	4 inches
Isopropylphenylcarbamate................	6	1.52*	2.28*	14.55	11.26
Isopropylphenylcarbamate................	3	4.23*	2.52*	13.59	10.93
2,4-dichlorophenoxyacetic acid..........	6	9.84	7.89	6.05*	6.22*
2,4-dichlorophenoxyacetic acid..........	3	9.41	10.02	8.34*	7.70*
2,4,5-trichlorophenoxyacetic acid........	6	8.75†	7.69	2.92*	3.18*
2,4,5-trichlorophenoxyacetic acid........	3	9.99	9.60	6.66*	5.28*
Untreated control.....................	....	11.55	8.56	12.72	12.32

* Weight of vegetative growth reduced below the untreated control (odds 99:1).
† Weight of vegetative growth reduced below the untreated control (odds 19:1).

6 mg. of isopropylphenylcarbamate, the coleoptiles were short and thickened at emergence. They became progressively darker blue-green in color during the first week following emergence. The tips of the coleoptiles then became brown. The necrosis progressed from the tip toward the base, and 23 days after treatment most had died without emergence of the plumule. In each pot one or two oat plants made some growth. This was apparently due to uneven distribution of the growth-regulator throughout the soil. The average weight of the aerial vegetative growth of these plants was

fect was apparent for 2 or 3 days following treatment. After the third day the color became noticeably darker green, and this color intensification continued for about 2 weeks, during which period little or no elongation or further production of leaves occurred. At approximately 2 weeks after treatment, the leaves started to turn brown at the tips. The leaves progressively dried from the tips, and nearly all plants were dead 23 days after treatment. Little difference was noted in the results produced by the 3- and the 6-mg. treatments. The weight of the aerial portions of the plants for

both rates was about one-third that of the untreated controls (table 1).

The two phenoxyacetic acid derivatives, whether applied at time of seeding or to young established plants, caused slight chlorosis and slight stunting of growth but failed to reduce the weight of vegetative growth of oats to a statistically significant degree (except 6 mg. of 2,4,5-trichlorophenoxyacetic acid). Isopropylphenylcarbamate produced no noticeable effect upon the soybeans and did not reduce the weight of vegetative growth (table 1). The high toxicity of the two phenoxyacetic acid derivatives upon soybeans is apparent from table 1.

EXPERIMENT 2

To determine whether the apparent lack of physiological effect of isopropylphenylcarbamate on soybeans was general for broadleaf species, this compound was applied at rates of 5–18 mg. to soil in which seeds of several species had been planted. Four-inch pots were used, each containing about 1 pound of soil, and the compound was applied in 25 ml. of water. Germination and development of the seedlings of soybeans, kidney beans, cowpeas, sunflowers, turnips, radishes, and sugar beets were not affected. Tomatoes and potatoes are broadleaf species which may have been affected. Soon after treatment of 10-day-old tomato plants, treated plants became noticeably taller than the controls, although no proliferation or epinasty was observed. Two months after treatment, these plants were about 50 inches in height, whereas the control plants were approximately 40 inches in average height. When 40 mg. of isopropylphenylcarbamate were applied to potatoes about 3 inches in height, the primary stem became stunted and rosette-like within two weeks after treatment. Fifty-one days after treatment the

rosette appearance of the primary stem persisted, but stems produced by the axillary buds appeared to be normal. The germination of buckwheat, *Fagopyrum esculentum*, was completely prevented by either 2 or 5 mg. of isopropylphenylcarbamate per 4-inch pot.

EXPERIMENT 3

This experiment was conducted to test the relative susceptibility of several cereal crops to the herbicidal activity of isopropylphenylcarbamate. Six cereals—

TABLE 2

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE UPON THE VEGETATIVE GROWTH OF SIX CEREAL SPECIES. GREEN WEIGHT (TAKEN 23 DAYS AFTER TREATMENT) OF TREATED POTS IS GIVEN IN PERCENTAGE OF THE UNTREATED CONTROLS, BASED UPON THE MEAN WEIGHT OF FOUR POTS PER TREATMENT. CONTROL EXPRESSED AS 100%

CROP	TREATMENT RATE	
	1.5 mg. per 4-inch pot	3.0 mg. per 4-inch pot
Oats (*Avena sativa*)........	30.1	22.7
Wheat (*Triticum vulgare*)..	23.2	28.7
Barley (*Hordeum vulgare*)..	61.7	42.2
Corn (*Zea mays*)..........	69.4	43.6
Rice (*Oryza sativa*).......	78.4	77.0
Millet (*Setaria italica*).....	165.6	97.8

oats, wheat, barley, corn, rice, and millet —were seeded in 4-inch pots. Pots of wheat, oats, barley, and rice were thinned to ten plants before treatment, millet to five plants, and corn to four plants. One and one-half or 3 mg. of isopropylphenylcarbamate were applied to the soil in 25 ml. of water 2 weeks after seeding, at which time the wheat, oats, corn, barley, and millet were about 2 inches tall and the rice about 1 inch tall. The pots were subirrigated subsequently. The fresh weight in grams of the aerial growth was taken 23 days after treat-

ment. Four pots were given each treatment.

No effect was apparent upon millet. Although the weight of the vegetative growth would indicate stimulation where 1.5 mg. were applied (table 2), it is likely that this was due to random error. All the other cereals were severely stunted by isopropylphenylcarbamate and the typical dark blue-green color appeared, which had been previously observed in oats following application of this compound. A few plants of all cereals except

ment the flooded rice had tillered profusely and was luxuriantly vegetative, with the leaves slender and dark green in color. The weight of the vegetative growth did not differ significantly from that of the untreated controls when harvested 23 days after treatment.

Further to test the effect of the compound upon rice, it was applied in aqueous solution to the irrigation water of rice about $3\frac{1}{2}$ months old at 10 or 30 mg. per 1-gallon glazed crock. Tillering was stimulated even in these older plants, but

TABLE 3

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE COMPARED TO SOME PHENOXYACETIC ACID DERIV-
ATIVES UPON THE PRODUCTION OF RICE GRAIN. THE MEAN WEIGHT (TAKEN AT MATURITY)
OF FOUR CROCKS OF FIVE PLANTS TREATED WITH 62.5 MG. IS GIVEN (IN GRAMS)*

| TREATMENT | YIELD OF GRAIN IN GRAMS | | | | |
| | Replications | | | | Mean |
	1	2	3	4	
Isopropylphenylcarbamate..................	12.4	9.2	4.8	8.4	8.7
Ammonium 2-methyl-4-chlorophenoxyacetate	52.5	55.4	48.7	28.9	46.4
Ammonium 2,4-dichlorophenoxyacetate......	46.1	54.0	61.7	48.1	52.5
1:1 mixture of isopropylphenylcarbamate and ammonium 2-methyl-4-chlorophenoxyace-tate.................................	17.3	24.5	18.3	11.6	17.9
Untreated control......................	68.8	52.7	56.7	89.2	66.9

* Minimum significant difference between treatment means is 5.4 gm. at the 5% level of significance and 7.6 gm. at the 1% level.

millet had died by the 23d day after treatment. Examination of the data in table 2 shows that oats and wheat were more inhibited in growth by isopropylphenylcarbamate than were barley, corn, or rice.

EXPERIMENT 4

Isopropylphenylcarbamate applied to the irrigation water of 30-day-old rice plants in flooded half-gallon crocks at 2.5–10 mg. per crock produced the typical dark-green color of foliage which characterized its effect upon younger nonflooded rice (expt. 3) and other cereals. However, 23 days after treat-

the tillers were somewhat stunted, maturity was delayed, and the panicles produced were small. The amount of grain was greatly reduced; the untreated control yielded 32.7 gm. per four pots, the 10-mg. treatment 22.2 gm., and the 30-mg. treatment 1.3 gm. Similar results were obtained from an experiment designed to compare isopropylphenylcarbamate with some of the phenoxyacetic acid derivatives. It is apparent from table 3, despite the recognized unreliability of greenhouse-yield results, that the former compound caused a greater reduction in grain yield of rice than the

phenoxyacetic acid derivatives against which it was compared.

EXPERIMENT 5

Two field trials were run in 1945 to test the herbicidal activity of isopropylphenylcarbamate upon plants under field conditions. Since insufficient amounts of isopropylphenylcarbamate were available for application at rates which were deemed desirable, it was decided to prepare a 1:1 mixture of this compound

The two preparations were applied to winter rye (sown the previous fall) on April 14, when the rye was about 1 foot tall. Rates calculated at 3, 10, and 20 pounds per acre of herbicide were applied to plots 10 feet square, which were arranged in a complete randomized block-field design with each treatment in quadruplicate.

Two weeks after treatment those plots which had received 10 or 20 pounds per acre of the mixture of 2,4-dichlorophe-

TABLE 4

A COMPARISON OF THE EFFECT UPON GRAIN YIELD (TAKEN AT MATURITY IN GRAMS) OF ISOPROPYL-
PHENYLCARBAMATE AND A MIXTURE OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLORO-
PHENOXYACETIC ACID APPLIED IN SAWDUST TO WINTER RYE 12 INCHES TALL*

TREATMENT†	RATE (POUNDS PER ACRE)	YIELD OF GRAIN IN GRAMS				
		Replications				Mean
		1	2	3	4	
2,4-dichlorophenoxyacetic acid..........	3	1001	658	820	954	858
2,4-dichlorophenoxyacetic acid..........	10	806	735	721	459	680
2,4-dichlorophenoxyacetic acid..........	20	632	721	490	684	631
Isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid (1:1 mixture)	3	530	228	475	457	422
	10	104	74	165	79	106
	20	43	0	0	0	11

* Minimum significant difference between treatment means is 53 gm. at the 5% level and 74 gm. at the 1% level of significance.
† The untreated control plots were lost during the threshing operation.

with 2,4-dichlorophenoxyacetic acid and compare the results with 2,4-dichlorophenoxyacetic acid alone. To facilitate a uniform distribution of these herbicides upon the soil, they were impregnated in an equal weight of oven-dry sawdust. This was accomplished by dissolving the 2,4-dichlorophenoxyacetic acid in an isopropyl alcohol–acetone mixture (1:1) and the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in isopropyl alcohol. The solutions were poured over the sawdust, which was then thoroughly stirred, and the solvent evaporated at 45° C.

noxyacetic acid and isopropylphenylcarbamate were stunted, dark green in color, and delayed in heading. By May 19 the plots treated with 2,4-dichlorophenoxyacetic acid alone were in full flowering stage, while plots which had received the mixture were stunted severely and had not started to head. The plots were harvested at maturity on July 27, and grain yield was taken in grams (table 4). Plots which had been treated with the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid were greatly reduced in grain yield. The reduction in yield increased directly

with increases of amount of the herbicide applied, with the differences between treatment rates highly significant statistically. Increases in the amount of

TABLE 5

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID UPON THE YIELD OF GRAIN (TAKEN AT MATURITY IN GRAMS) OF OATS. FIGURES REPRESENT THE MEAN OF FOUR PLOTS, 6×8 FEET*

Rate of Application (pounds per acre)	2,4-Dichlorophenoxyacetic acid	Mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid (1:1)
First application (planting)		
3	188	197
10	106	50
20	70	8
Second application (2 inches tall)		
3	195	83
10	92	8
20	55	7
Third application (prebooting)		
3	317	275
10	221	167
20	184	96
Fourth application (heading)		
3	320	295
10	315	267
20	386	312
Untreated controls		
0	316	370
0	327	360

* Minimum significant difference between treatment means is 53.2 gm. at the 5 per cent level of significance and 70.7 gm. at the 1% level.

2,4-dichlorophenoxyacetic acid applied likewise resulted in smaller yields, but even the 20-pound per acre application was less effective in reducing yield of grain than the 3-pound per acre application of the mixture. It is readily apparent that isopropylphenylcarbamate was the more active portion of the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid.

Similar results were obtained when the same treatments were applied to seedling oats. Applications were made at different stages of development—seeding, 2 inches tall, prebooting, and heading—to plots 6×8 feet in size, arranged in a complete randomized block design replicated four times. The data tabulated in table 5 show that the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid was much more active in inhibiting grain yield of oats than 2,4-dichlorophenoxyacetic acid. Again the more active portion of the mixture was the isopropylphenylcarbamate. Applications at planting time proved slightly less effective than applications to oat seedlings about 2 inches in height (table 5). Both early applications were more effective than the one made to plants about a foot in height. Very little effect was obtained from applications after the oats had reached the heading stage of development.

Spray trials

Several experiments were conducted to determine the effectiveness of spraying as a method of applying isopropylphenylcarbamate. The low solubility of isopropylphenylcarbamate in water, about 250 p.p.m. at 25° C., proved too low to make aqueous sprays practicable. Isopropylphenylcarbamate is insoluble in oil. However, it may be dissolved in twice its weight of tributylphosphate,

and the resulting solution is oil miscible in all proportions. Oil sprays in the greenhouse were applied in a spray chamber with an area of 0.5 square yards and 4 feet deep, with a De Vilbiss atomizer operated at a pressure of 15 pounds per square inch. Sprays in the field were applied with a De Vilbiss CV type nozzle, operated at 15 pounds pressure per square inch from a pressure cylinder. Field plots were protected from the effect of wind during the spray operation by means of canvas-covered frames about 4 feet high.

rate of 2 or 10 pounds per acre in 10 ml. of tributylphosphate-oil per square yard to the rye when it reached the prebooting stage of development. The isopropylphenylcarbamate at 10 pounds per acre caused rapid death of all plants in plots to which it was applied, and no grain developed (table 6). The 2,4-dichlorophenoxyacetic acid was much less effective in herbicidal action.

Discussion

Although largely without effect upon broadleaf species, isopropylphenylcarba-

TABLE 6

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN TRIBUTYLPHOSPHATE-OIL SPRAY TO WINTER RYE IN THE PREBOOTING STAGE OF DEVELOPMENT. YIELD OF GRAIN IN GRAMS WAS TAKEN AT MATURITY

TREATMENT*	RATE (POUNDS PER ACRE)	YIELD OF GRAIN IN GRAMS				
		Replications				Total
		1	2	3	4	
Isopropylphenylcarbamate.............	2	154	94	161	132	541
Isopropylphenylcarbamate.............	10	0	0	0	0	0
2,4-dichlorophenoxyacetic acid.........	2	95	136	216	124	571
2,4-dichlorophenoxyacetic acid.........	10	70	82	67	105	324

* The untreated control plots were lost during the threshing operation.

The results of several oil-spray experiments in the greenhouse were inconclusive. Rates of isopropylphenylcarbamate up to 0.5 gm. per square yard, applied in 10 ml. of tributylphosphate-oil per square yard, did not inhibit vegetative growth of young oats, wheat, rice, barley, corn, or millet to a statistically significant degree. Indications were obtained that similar applications to oats in the prebooting stage of development greatly reduced grain yield.

Isopropylphenylcarbamate was compared with 2,4-dichlorophenoxyacetic acid in a field experiment with winter rye. These compounds were applied at a

mate proved to be a more effective herbicide upon several cereals than some halogenated phenoxyacetic acids. The fact that isopropylphenylcarbamate was not effective on *Setaria italica* may indicate that its herbicidal properties are not general for the grasses and for related families. However, the results indicate a possible avenue of approach to the control of weedy grasses, for which purpose the phenoxyacetic acid series of compounds has not been promising.

Application of isopropylphenylcarbamate via the soil was more successful in the present experiments than was its application in oil-sprays to aerial plant

parts. The high effectiveness of oil-spray applications upon winter rye indicates that the spray method of application may be useful under field conditions.

Further studies of isopropylphenylcarbamate and similar compounds, as well as methods of application, are in progress.

Summary

1. Comparisons have been made of the herbicidal effectiveness of isopropylphenylcarbamate and some phenoxyacetic acid derivatives.

2. While isopropylphenylcarbamate applied in aqueous solution to soil at rates of $1\frac{1}{2}$–6 mg. per 4-inch pot severely stunted or killed seedling oats, wheat, corn, barley, and nonflooded rice, similar applications of the phenoxyacetic acid derivatives did not cause statistically significant inhibition of growth.

3. Isopropylphenylcarbamate applied to the soil was ineffective in herbicidal activity upon soybeans, kidney beans, cowpeas, sunflowers, radishes, turnips, and sugar beets. It possibly had a slight stimulatory action on tomatoes and appeared to cause stunting of young potatoes, an effect which was subsequently outgrown. The germination of buckwheat was completely prevented by isopropylphenylcarbamate.

4. Isopropylphenylcarbamate proved to be a highly effective herbicide upon field-grown oats and rye when applied in sawdust to the soil at seeding time or to seedling plants. It had a lesser inhibiting action upon older plants.

5. The results of applications of isopropylphenylcarbamate in oil sprays were inconclusive. Although the vegetative growth of greenhouse cereals was not inhibited, field-grown winter rye was killed.

6. Isopropylphenylcarbamate is a highly selective herbicide for certain cereals, but whether its toxicity is general for the Graminae and related families is not known. The possible use of this compound as a herbicide for the control of weedy grasses is suggested.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

I. SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

Attention has been directed toward the possible herbicidal use of certain substances known to possess regulatory activity affecting the amount and type of growth. Such use was suggested as early as 1941 by E. J. KRAUS. Since then, similar suggestions have been made independently in the literature by a number of authors (1, 2, 5, 6, 10, 11). These and more recent reports (3, 12, 14, 15, 18) have centered attention especially on chlorinated derivatives of phenoxyacetic acid and on naphthoxyacetic acid. The susceptibility of young broadleaf plants to herbicidal treatments with growth-regulators appears, in general, to be high. Cereal crops are affected, but they appear only slightly so at most levels of treatment that are inhibitory or toxic to broadleaf plants.

Herbicidal effects have resulted when these chemicals have been applied as sprays at a rate of the order of 1 pound per acre or as soil applications, with some solid inert diluent to obtain an active-ingredient rate of the order of 2 pounds per acre. The latter method is highly interesting when considered with respect to common farming equipment and practices. Accordingly, it seemed desirable to attempt to investigate further the specific effects of some such chemical regulators on some common broadleaf and cereal plants. Such tests were conducted with young vegetative plants during the third through the sixth weeks of growth or some part of that period, unless otherwise specified.

The nutrient-solution culture technique was selected as one means of investigation in view of the possibility that growth-regulators might be employed as soil (root) treatments and because this technique allows accurate observation of the responses of root systems, as well as shoot responses. It was also possible by means of this technique to conduct tests dealing with treatment presentation time and to obtain some information about the modifying influence of soil on the effectiveness of known amounts of chemical growth-regulator. A number of investigators have previously used the nutrient-solution technique to investigate some of the effects of growth-regulating substances in relation to nutrition and growth of kidney beans and ornamental plants (4, 9, 17).

Methods and materials

Large numbers of Silver King (dent) corn, Illini soybeans, Marquis wheat, Victory oats, Onsen rice, and red kidney bean seeds, which were selected for soundness and uniformity, were planted in the greenhouse in flats containing gravel which was kept moist with HOAGLAND's standard nutrient solution (7). When seedlings had attained the desired size, they were transferred to 6700-ml. glazed-clay nutrient-solution containers

and grown for 1 week to allow seedlings to become established before treatment. All endosperm or seed residues were removed from the cereal seedlings at the time of their transfer into solution cultures.

The standard nutrient solution used in these tests (except in the test on rice) contained chemically pure chemicals in the following concentrations: 0.005 M. monopotassium acid phosphate, 0.001 M. ammonium dihydrogen phosphate, 0.005 M. calcium nitrate, 0.005 M mag-

A minimum continuous photoperiod of 12 hours was maintained during the winter months by means of a line of five 150-watt Mazda lamps equipped with 14-inch reflectors, suspended at intervals of 4 feet along the center of the 19×3.5-foot bench on which the cultures were located in randomized blocks.

Experimental results

EXPERIMENT I

In preliminary observational tests, 10-day-old soybean and kidney bean plants

FIG. 1.—Soybean plants grown for 9 days in nutrient solution (C), and in similar solution containing 2,4-dichlorophenoxyacetic acid at the following concentrations: *1*, 0.66; *2*, 0.33; *3*, 0.16; *4*, 0.08; and *5*, 0.04 p.p.m.

nesium sulphate, 0.00012 M. ferric citrate, and minor elements in the amounts recommended by HOAGLAND (7). The nutrient solution was prepared with distilled water, and the pH of the solution was adjusted initially to 6.0–6.2 with sodium hydroxide. All solutions and treatments were renewed weekly, except where different management in a test is otherwise specified.

It was normal procedure to aerate the cultures for at least 1 hour daily, except-ing rice cultures, which were not aerated.

were exposed for 2 weeks to nutrient solutions containing 0.04–3.3 p.p.m. of 2,4-dichlorophenoxyacetic acid. Cowpeas were treated with 0.08–0.6 p.p.m. of this growth regulator. Treated plants developed no uniform or distinct symptoms of stem curvature or epinasty. By the 9th day it was apparent that shoot growth of all plants was decreased with 0.08 p.p.m., while growth of axillary buds from the cotyledonary nodes was stimulated by 0.04 p.p.m. After the 10th day of treatment, soybean and kidney bean plants

were permanently wilted in cultures which contained 0.6 p.p.m. or more. Though shoots of cowpea plants did not wilt during 2 weeks of continuous exposure, their appearance was distinctly chlorotic and unhealthy in cultures which contained 0.3 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. The increase in dry weight of root, shoot, or total plant was inversely related to concentrations of growth-regulator. The growth of plants treated with 0.08 or 0.16 p.p.m. was reduced to approximately 75 and 50 per cent, respectively, of that of untreated plants (fig. 1).

In cultures which contained different volumes of solution (1700, 3600, and 6700 ml.) and equal weights of 2,4-dichlorophenoxyacetic acid, the amount of cowpea growth which occurred during a 2-week period suggested that the effect of a given amount of the regulator was influenced by the concentration presented (fig. 2). Representative plants are shown in figure 3. Consistently, the earliest and most outstanding symptom which appeared on treated plants was swelling of the lower hypocotyl and parts of roots. This was evident on the 3d day in solutions containing more than 0.04 p.p.m. and became more marked until, by the 7th day, the surfaces of hypocotyls of kidney beans were corky and
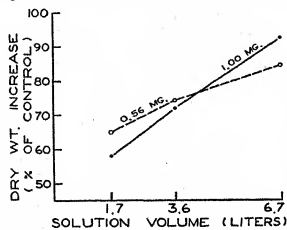
FIG. 2.—The influence of concentration on the effect of 2,4-dichlorophenoxyacetic acid on the growth of cowpeas. Labels on curves indicate weight of regulator per culture vessel. The final dry weight of untreated plants is valued as 100 per cent.

FIG. 3.—The influence of concentration and amount of 2,4-dichlorophenoxyacetic acid on growth of cowpeas during a 2-week test period.

TREATMENTS

Number	5	3	1	C	2	4	6
Regulator (mg.)	0.55	1.0	2.0	0.0	2.0	1.0	0.55
Culture volume (liters)	6.9	6.9	6.9	3.6	3.6	3.6	3.6
Regulator (p.p.m.)	0.08	0.15	0.29	0.0	0.56	0.28	0.15

split by longitudinal fissures in all concentrations of 0.6 p.p.m. or less. In respective order, soybeans and cowpeas showed this response in lesser degrees.

Production of thick, non-elongated roots from the lower hypocotyl of kidney

fasciated sheets of tissue were oriented mainly in four radial planes along the hypocotyl, the general appearance of which was similar to that previously noted when various growth-regulators were applied to shoots of plants (8, 15)
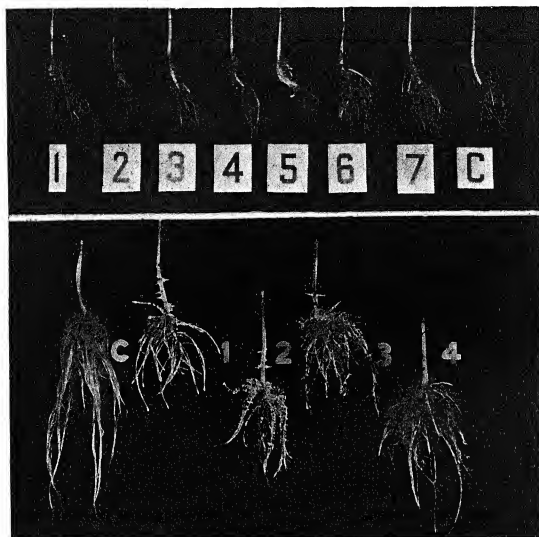
Figs. 4, 5.—Fig. 4, the effect of 2,4-dichlorophenoxyacetic acid on hypocotyls and roots of kidney bean plants. Treatments: 1, 3.30; 2, 1.70; 3, 0.60; 4, 0.33; 5, 0.16; 6, 0.08; 7, 0.04 p.p.m. of the regulator; C, control in nutrient solution only. Fig. 5, hypocotyls and root systems of cowpea plants grown in nutrient solutions containing 2,4-dichlorophenoxyacetic acid. Treatments: 1, 0.60; 2, 0.33; 3, 0.16; 4, 0.08 p.p.m. of the regulator; and C, control in nutrient solution only. Decreased root elongation in 4, decreased elongation and swelling of root tips in 3, marked swelling of lateral roots in 2, general lack of growth in 1, and progressive decrease of adventitious root growth from hypocotyls in 1–4.

beans and, to lesser degree, in soybeans and cowpeas was stimulated by 0.04 p.p.m. or more. Such roots on kidney beans often became fasciated. These characteristics became more striking as the concentration was increased from 0.16 to 0.6 p.p.m. Adventitious roots or

(figs. 4 and 5). Growth was so completely arrested by more than 0.6 p.p.m. of 2,4-dichlorophenoxyacetic acid that these symptoms were expressed in only a slight degree prior to the death of the plants.

Root systems of plants grown in

treated solutions were somewhat darkened, possibly because of tissue deterioration or death. Elongation of primary and lateral roots, almost lacking at 0.33 p.p.m., was inhibited in proportion to regulator concentration. Distinct suppression of root elongation, accompanied by thickening, especially in the region 2–5 mm. behind root tips, was evident on plants treated for 4 days with 0.16 p.p.m., and by the 12th day in 0.04–0.08 p.p.m.

Experiment II

In a second test on 17-day-old soybeans, triplicate cultures, each of which contained four plants, were treated with 2,4-dichlorophenoxyacetic acid from 1 to 60 p.p.m. The time required to kill plants was inversely related to the concentration of regulator in the nutrient (fig. 6). Death of more than one-half of the plants in cultures containing 1 p.p.m. and of all plants in cultures with more than 2 p.p.m. had occurred by the end of the 8th day of treatment. Untreated plants were growing normally when the test ended on the 10th day. For any one concentration the time required to kill all plants in the triplicate cultures varied not more than 1 day from the average time indicated for that concentration.

During the test period, true stem curvatures or epinastic responses occurred in a small proportion of the treated plants. The frequency of this response was erratic and was not clearly relatable to the concentration applied. The most typical symptom, namely, general wilting of leaves and shoots, appeared after periods of treatment which varied inversely with the concentration. The foliage was strongly wilted after 18 hours in nutrient solutions containing 25 p.p.m. or more. The primary leaves wilted first, then wilting rapidly pro-

gressed upward to involve all leaves and the entire shoot.

As soon as all plants in any culture were dead, shoots of the plants were divided into two fractions—new shoot and old shoot or the shoot up to the primary leaf node plus the primary leaves—and dry-weight data were obtained (table 1). Although some plants at the lower concentrations did not die for a number of
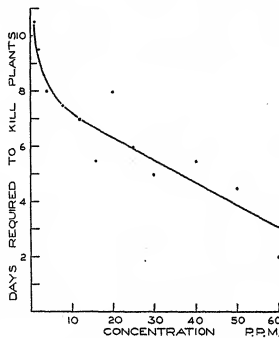
Fig. 6.—Length of time to death after addition of 2,4-dichlorophenoxyacetic acid to 17-day-old soybean plants in nutrient solution. Time shown is average for triplicate cultures, each having four plants.

days, the weight data indicate that significant increase in plant dry weight did not occur in concentrations above 2 p.p.m. Irregular growth in cultures containing 1 or 2 p.p.m. resulted in plant dry-weight increases of 10 per cent compared to control plants. There was no indication of increase in dry weight of roots with more than 2 p.p.m. The 50 per cent increase in weight of roots in 1 p.p.m. was not due to normal development. In cultures which contained 4 p.p.m. or less, any visible new shoot growth, which appeared before the death of plants, was

much stunted and irregular. The major increase in plant weight appeared attributable to somewhat irregular development of hypocotyls, which occurred on plants grown in concentrations as high as 25 p.p.m. Growth of old portions of the shoot, new shoot, and roots, respectively, were increasingly inhibited by 2,4-dichlorophenoxyacetic acid.

No visible symptoms had appeared in 48 hours after the plants were initially placed in 1 p.p.m., but the lower leaves of some plants in 5 p.p.m. had developed a slightly lighter color than those of untreated plants, and distinct stem curvature was evident near the shoot apex. These responses were distinct on the 3d day, when the lower leaves of some

TABLE 1

THE INCREASE IN DRY WEIGHT OF 17-DAY-OLD SOYBEAN PLANTS AFTER ADDITION OF 2,4-DICHLOROPHENOXYACETIC ACID TO THE NUTRIENT SOLUTION. MEAN WEIGHTS SHOWN REPRESENT FOUR PLANTS

REGULATOR (P.P M)	FINAL DRY WEIGHT (MG.)*				PERCENT INCREASE IN WEIGHT†			
	Old shoot	New shoot	Roots	Total plant	Old shoot	New shoot	Roots	Total plant
0	720± 60	2330±300	370±60	3420±520	100	100.0	100	100.0
1	470± 90	390± 30	240±70	1100±200	20	6.0	46	11.0
2	500±120	380± 10	160±60	1040±200	29	5.5	12	9.0
4	460± 50	370± 40	90±20	920±100	16	5.0	0	4.0
8	460± 30	340± 20	70±10	880± 20	16	3.5	0	2.5
12	490± 40	350± 20	90±20	930± 80	26	4.0	0	4.5
16	430± 30	290± 70	70±10	790± 70	7	1.0	0	0.0
20	480± 60	270± 50	90±10	830±140	23	0.0	0	1.0
25	470± 50	310± 50	70±10	830±120	19	2.0	0	1.0
30	420± 50	200± 20	70±10	700± 70	3	0.0	0	0.0
40	380± 40	240± 30	60±10	690± 60	0	0.0	0	0.0
50	370± 10	260± 20	100±20	730± 40	0	0.0	0	0.0
60	410± 20	330± 10	110±10	850± 50	0	3.0	0	1.5
Initial weight (mg.)	410	270	130	810	......	......	......	......

* Mean dry weight and maximum variation are shown.

† The increase in dry weight of untreated plants or plant parts is valued as 100 per cent.

EXPERIMENT III

Three-week-old kidney beans, which had two trifoliate leaves unfolded on shoots 8–10 inches long above the primary leaf node, were exposed for 2 and 3 days to 1 and 5 p.p.m. of 2,4-dichlorophenoxyacetic acid. After the desired presentation time had elapsed, the plants were removed from each of the duplicate cultures and placed in normal nutrient solution. Observations of symptoms and responses were made during the succeeding 14 days.

plants which had remained in 1 p.p.m. were chlorotic. At this time the older leaves on plants in 5 p.p.m. were distinctly wilted.

During the period from the 3d through the 6th day, all treated plants appeared to make some recovery in the plain solutions. All lower leaves of plants treated with 1 p.p.m., especially those exposed only 2 days, became more green. Though the lower leaves of plants treated with 5 p.p.m. were dry or dead on the 6th day, the shoots had recovered from the curva-
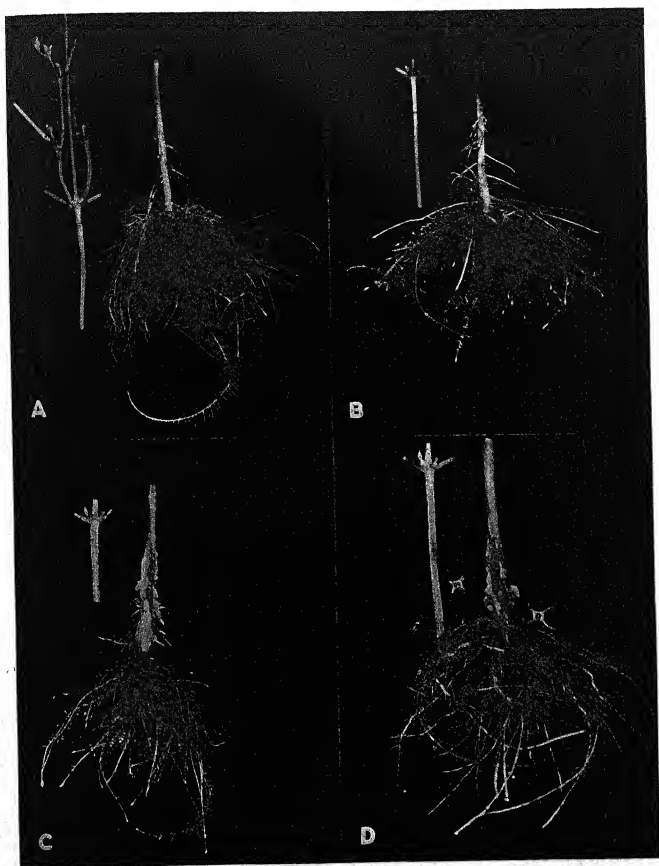
Fig. 7.—Root systems, hypocotyls, and lower shoots of kidney bean plants variously treated with 2,4-dichlorophenoxyacetic acid in nutrient solutions. Treatments initiated on the 15th day of growth and plants photographed 15 days later. *A*, plant not treated; *B*, 2 days of exposure to 1 p.p.m.; *C*, 2 days of exposure to 5 p.p.m.; *D*, 3 days of exposure to 5 p.p.m. Progressive increase in inhibition of elongation and branching of roots; increase in swelling of hypocotyl and production of adventitious roots as treatment intensity was increased; maximum root-tip swelling on plants exposed for 2 days; and axillary bud proliferation at 5-p.p.m. treatment.

ture response. Hypocotyls were swollen and the meristem regions of roots and shoots were slightly swollen.

In 8–10 days after initial application of 3-day treatments with 5 p.p.m. of 2,4-dichlorophenoxyacetic acid, the hypocotyls were much swollen, and terminal, and some lateral shoot meristems

sulted in fasciation of such roots to form four vanes or sheets of tissue extending along and radially out from the ruptured hypocotyls. These symptoms and typical shoot-system responses are shown in figures 7 and 8.

At the end of the test period the amount of growth of shoots of plants ex-

FIG. 8.—Upper shoots of kidney bean plants variously treated with 2,4-dichlorophenoxyacetic acid, 15 days after initiation of treatments. A, 3-day exposure to 5 p.p.m.; B, 2-day exposure to 5 p.p.m.; C, untreated. Decreased shoot growth and etiolated internodes, suppression of normal growth, and swelling of terminal and lateral meristems as a result of treatments.

were "barrel-like" hypertrophies, tipped by stunted, mottled, dark-green leaves. These symptoms developed less strongly in plants treated with 1 p.p.m. or for 2 days with 5 p.p.m. In proportion to the intensity (concentration × time) of regulator treatment applied, production of adventitious roots from the swollen lower hypocotyl region was greatly stimulated. Three days of exposure to 5 p.p.m. re-

posed for 1 day to 1 p.p.m. appeared equal to that of control plants. Relatively little growth of shoots had occurred in plants treated 3 days with 5 p.p.m., and intermediate treatments caused visible inhibition. Untreated plants were flowering profusely at the end of the test period, whereas the 3-day treatment with 5 p.p.m. delayed or completely curtailed reproductive development. All treat-

ments caused degrees of this response which were proportional to treatment intensity.

EXPERIMENT IV

Preliminary tests were conducted on wheat and corn plants in 1700-ml. cultures during the period from the 2d through the 5th weeks of growth. Wheat cultures, each containing ten plants,

leaves of these plants was observed. Though both corn and wheat plants growing in 8 p.p.m. were alive after 1 week of treatment, control plants were visibly further advanced than all plants treated with more than 0.5 p.p.m. Symptoms were evident on roots of treated plants by the 4th day (figs. 9 and 10). Representative wheat cultures treated for 20 days are shown in figure 11.

TABLE 2

FINAL DRY WEIGHT OF WHEAT AND CORN PLANTS GROWN IN NUTRIENT SOLUTIONS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID. AVERAGE WEIGHTS SHOWN ARE FOR TRIPLICATE CORN AND DUPLICATE WHEAT CULTURES TREATED CONTINUOUSLY FROM THE 2D THROUGH THE 5TH WEEKS OF GROWTH

| REGULATOR (P.P.M) | WEIGHT PER CORN PLANT | | | | | | WEIGHT PER WHEAT PLANT | |
| | Shoots | | Roots | | Total plant | | | |
	Mg.	%	Mg.	%	Mg.	%	Mg.	%
0.0	160.6	100	47.6	100	208.3	100	226	100
0.5	61.6	39	31.6	67	93.2	45	......	......
1.0	41.0	26	25.6	54	66.6	32	......	......
2.0	35.3	22	23.3	49	58.6	28	......	......
3.0	......	......	......	......	......	......	87	39
4.0	36.3	23	17.6	37	53.9	26	76	34
6.0	......	......	......	......	......	......	61	27
8.0	33.6	21	12.6	27	46.2	22	51	23
Initial weight (mg.)	33.0	......	8.5	......	41.5	......	......	......
	Minimum significant differences—mg.							
At 99:1 odds	23.8		24.1		9.7		......	
At 19:1 odds	17.1		17.3		7.0		33	

were treated with concentrations of from 3 to 8 p.p.m. Concentrations ranging from 0.5 to 8.0 p.p.m. were applied to triplicate corn cultures, each containing three plants. At the start of the test period the shoots of wheat plants were 6 inches long; and those of corn were 8–10 inches in length, with the fourth leaf just visible.

During the test periods no sign of any shoot curvature or growth response of

At the end of the test period, normal development of roots had been almost completely inhibited by 4 p.p.m. or more of the regulator. The symptoms which developed on roots of these plants were similar to those observed on broadleaf crops with regard to type and time of expression. Crowns of wheat plants were distinctly swollen in cultures containing 3–6 p.p.m. Maximum abnormal development of this type appeared to occur at

less than 6 p.p.m., possibly because higher concentrations almost completely arrested all growth (fig. 12). Stimulation of the growth of adventitious roots from the crowns of treated corn plants was less evident than for wheat plants.

parts of the youngest leaves remained green at 4 p.p.m.

The lowest 2,4-dichlorophenoxyacetic acid concentrations tested, namely, 3 and 0.5 p.p.m. on wheat and corn respectively, resulted in highly significant re-
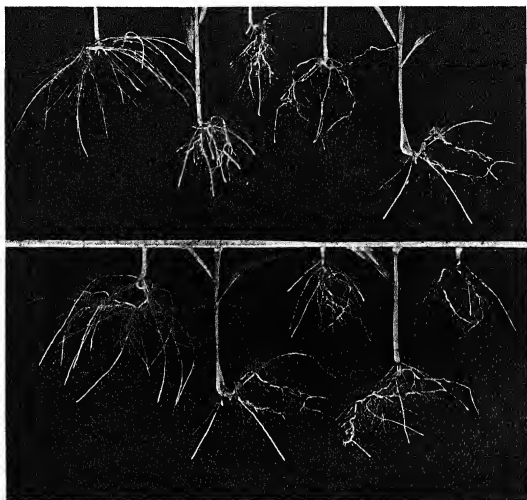
FIGS. 9, 10.—Fig. 9, roots of 26-day-old corn plants after 4 days of growth in nutrient solution containing (left to right) 0.0, 0.5, 1.0, 2.0, and 4.0 p.p.m. of 2,4-dichlorophenoxyacetic acid. Marked inhibition of growth caused by more than 2 p.p.m.; increased inhibition as concentration increased; root tips swollen; and general lack of development or stimulation of adventitious roots. Fig. 10, root systems of corn plants after 4 days in nutrient solution containing (left to right) 0.0, 4.0, 8.0, 15.0, and 25.0 p.p.m. of 2,4-dichlorophenoxyacetic acid.

The growth of shoots of both crops was markedly less in all concentrations tested. After the first week of the test period, shoots did not appear to increase in length, and, progressing upward on the shoot, leaves dried from the tip toward the base. At the end of the test, leaves of plants treated with 6 p.p.m. or more were completely dead, and only

ductions of 60 and 55 per cent in the dry weight of the plants (table 2). Increase in the concentration of the regulator to 8 or to 1 p.p.m. or more, respectively, resulted in significantly less average final dry weight of wheat and corn plants. Compared to untreated plants, the final dry weight of wheat plants grown in 6–8 p.p.m. was 25 per cent, while an ap-

FIGS. 11, 12.—Fig. 11, shoots of wheat plants in cultures containing 3–8 p.p.m. of 2,4-dichlorophenoxy-acetic acid after 20 days of continuous treatment. Numbers on pots indicate p.p.m. concentrations. Shoots are partly dry in the 3-p.p.m. culture, and in other treated cultures plants are dead. Fig. 12, representative wheat plants after 20 days of growth in cultures which contained 0, 3, 4, 6, and 8 p.p.m. of 2,4-dichlorophenoxy-acetic acid. Inhibition of growth in proportion to concentration; marked swelling at the crown region; some increase of adventitious root growth, which was soon arrested; treated roots dark in color.

proximately equal degree of reduction of weight of corn plants occurred in 4 p.p.m.

The roots of corn plants continued to increase in dry weight in solutions containing more than 1 p.p.m., although shoot growth was completely arrested (fig. 13). In normal plants the weight of shoots accounted for the major portion of the total increase in plant weight, whereas the major part of the increase in weight of corn plants grown in more than 0.5 p.p.m. was due to growth of roots.

Judging from visual observation, this relation did not appear to hold with

FIG. 13.—The increase in dry weight of wheat and corn plants during the 2d through the 5th weeks of growth in nutrient solution containing 2,4-dichlorophenoxyacetic acid.

treated wheat plants. The fact that growth of shoots of corn did not parallel growth of roots prior to death of plants, as well as the appearance of the root systems, suggests that the root tissues which did develop were abnormal and did not function normally.

Discussion

Employing sand cultures, LAUDE (9) found that treatment with low concentrations of indoleacetic acid resulted in an increase in the weight of kidney bean plants when potassium in the nutrient solution did not limit growth. The roots of plants were more fibrous and increased approximately 50 per cent over normal weight when similar concentrations of

naphthalene acetamide were applied in the nutrient solution, which responses confirmed earlier observations by other investigators (13). At this concentration the growth of shoots was significantly reduced. Using a comparable technique with marigolds, SWARTZ (17) found that 0.01 mg. of naphthalene acetic acid per liter of nutrient solution decreased the weight of shoots and of the total plant, but only slightly decreased the weight of roots. Such treatments did not inhibit cosmos plants, and 0.1 mg. per liter did not result in significant change in the dry weight of chrysanthemums. Visible effects on the growth habit of the plants or on floral initiation were lacking.

In the present investigations, when 0.5–1.0 p.p.m. of 2,4-dichlorophenoxyacetic acid was supplied in solution, the increase in the dry weight of all parts of soybeans was significantly reduced, and the habit of the plants was changed. It was indicated in these tests, and confirmed in more detailed studies to be described in later papers of this series, that less inhibition of increase resulted in the weight of root than of shoot or total plant when several plants were treated with 2,4-dichlorophenoxyacetic acid or similar substituted phenoxyacetic acid derivatives. Floral initiation in kidney beans was retarded by low-intensity treatments (for 2 or 3 days at 1 or 5 p.p.m.) with this regulator.

Morphological changes of parts of shoots and roots of crop plants did result from treatment with 2,4-dichlorophenoxyacetic acid. Continuous treatments with 0.25–60 p.p.m. of this acid appeared to stimulate such responses in shoots less than milder discontinuous treatments. Curvatures of stems and formation of shoot meristem hypertrophies were infrequent in these tests, and these occurred erratically with respect to treat-

ment concentration. The most consistent response of shoots was complete cessation of growth, which, in proportion to the concentration of regulator applied, was soon followed by wilting and drying of parts of plants. Though swelling of the crown region of cereals was distinct, in response to equivalent treatments relatively more obvious changes occurred in the type of growth of shoots of the broadleaf crops.

Morphological changes in the root systems of both groups of plants were the most striking and consistent responses which developed. In this respect and also in regard to the relative amount of growth of roots, the results in these tests with 2,4-dichlorophenoxyacetic acid most nearly correspond to those described by HAMNER (4), who used alphanaphthalene acetamide on kidney beans in nutrient sand cultures. In addition to decreasing the amount of growth of shoots and inhibiting expansion of leaves, which became curled and thickened, such treatment with 0.34 mg. of the latter regulator per liter resulted in short, stubby roots of approximately twice normal diameter, the increases in the weight of which were up to 37 per cent more than normal. Production of secondary roots occurred from about 0.6 mm. back from the apical meristem, where xylem and phloem elements were found to have matured early. Such responses were not described for treatments with phenylacetic acid, which consistently decreased the dry weight of bean plants and caused greater reduction in weight of shoots than of roots.

The much-inhibited, nonfibrous, nonelongated, discolored, bulbous-tipped root systems, with abnormal numbers of adventitious roots, which were developed when species of both groups of plants were treated with 2,4-dichlorophenoxy-acetic acid in these tests, much resembled those of beans grown in alpha-naphthalene acetamide. At the same time, their dry weight was much decreased from normal. In contrast to increased weight of roots caused by the latter regulator and the lack of marked effect of treatment with phenylacetic acid, 2,4-dichlorophenoxyacetic acid treatments significantly reduced the dry weight of the root systems of all species tested. The morphological responses of these plants to treatments with 2,4-dichloro-phenoxyacetic acid very much resembled the telemorphic responses of roots of sweet peas which were observed to follow treatment of the shoots with 4-chlorophenoxyacetic acid (1).

The growth of cereals, though relatively less affected than that of broadleaf crops (19), was somewhat modified in form and distinctly reduced by treatment with low concentrations of 2,4-dichlorophenoxyacetic acid. This was interesting, considering the emphasis in the literature on the differential effect of this compound. The results of these tests and of other more extensive tests, in which similar responses occurred, to be reported in later papers of this series, in part parallel those recently reported concerning the growth of several lawn grasses (12). Detrimental effects of the acid on these grasses were shown to be temporary in the soil and to last not more than 5 weeks after treatments up to 3 pounds per acre had been applied. Though growth was significantly stunted for periods of from 5 to 14 weeks following the applications, depending on the rate of treatment, established plants were not killed by $\frac{3}{4}$–3 pounds per acre of 2,4-dichlorophenoxyacetic acid. Except with the most rigorous treatments, they recovered, to make normal growth later.

Like lawn grasses, the cereal crops re-

mained alive at the end of test periods in nutrient solutions containing amounts of regulator which during equal or shorter periods proved lethal to the broadleaved crops. Distinct differential effects could result if treatment were temporary, allowing the more resistant cereals to recover later and to continue normal growth.

Summary

Young vegetative red kidney bean, soybean, cowpea, wheat, and corn plants were grown for from 2 to 3 weeks in nutrient-solution cultures which contained various concentrations of 2,4-dichlorophenoxyacetic acid.

This growth-regulator, in proportion to the concentration supplied, proved toxic to all species tested, reducing or inhibiting growth and causing distinct morphological changes in these plants.

Cereal crops were slightly more resistant than broadleaved crops. The growth of broadleaved plants was decreased by concentrations as low as 0.15 p.p.m., and soybeans were dead after exposure to from 1 to 5 p.p.m. for from 8 to 10 days. Cereal plants were alive after 20 days in nutrient solutions containing 3 p.p.m. or more, though their dry weight was significantly decreased by the lowest concentrations in which they were grown, namely, 0.5 and 3.0 p.p.m. The broadleaved plants appeared to have resistance to the regulator, increasing slightly in the following order: kidney bean, soybean, and cowpea. Wheat appeared more resistant than corn.

The dry weight of the shoots of plants was decreased slightly more than that of roots by low concentrations, but the modification of the habit of root systems was more consistent and distinct.

In proportion to concentration, 2,4-dichlorophenoxyacetic acid decreased elongation and branching of roots, caused swelling of root tips, swelling of some shoot meristems, and thickening of the hypocotyls of broadleaved plants, from which the growth of adventitious roots was stimulated by low concentrations. Few epinastic or stem-curvature responses occurred on shoots, which more typically wilted. The morphological responses of the shoots of broadleaved plants were more striking than those of cereals.

Wilting and drying of leaves and swelling of the crown region, from which the growth of adventitious roots was stimulated, were noted on shoots of cereals, the root systems of which responded quite similarly to those of broadleaved plants.

Low-intensity treatments retarded floral initiation of red kidney beans.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

II. THE INFLUENCE OF PRESENTATION TIME[1]

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Introduction

In preliminary tests (2) it was observed that, when low concentrations of 2,4-dichlorophenoxyacetic acid were added to nutrient solutions in which species of broadleaf and cereal crops were developing, growth was arrested, or marked morphological changes ensued, especially in the root systems. In several quantitative tests, plants were exposed to 2,4-dichlorophenoxyacetic acid for various periods of time for comparison with continuous treatment with that compound.

After such exposure they were removed to cultures free of growth-regulator, and observations of their development were continued. Such tests in which plants were subjected to known concentrations of growth-regulators for limited periods of time made possible more precise study of the effects of treatment intensity (concentration × presentation time).

The materials and methods employed in conducting these presentation-time studies were similar to those used in the preliminary tests (2).

Experimental results

Soybean plants, 2 weeks old, were treated for 1, 2, or 7 days with 1, 5, or 20 p.p.m. of 2,4-dichlorophenoxyacetic

acid, after which they were transferred to normal nutrient solutions until the end of the 5th week. Similar plants were also treated continuously with each of these concentrations of the regulator from the 2d through the 5th weeks of growth.

Wilting of plants did not result from 2 days of exposure to 5 p.p.m. or less, and

days or more to 5 p.p.m. or higher concentrations. These plants did not regain normal color after transfer to plain nutrient solutions, and drying or death of such leaves progressed upward on the plants.

No shoot proliferations were noted on any of the treated plants. Typical swell-

TABLE 1

THE INCREASE IN DRY WEIGHT OF SOYBEAN PLANTS WHICH OCCURRED DURING THE 3D THROUGH THE 5TH WEEKS OF GROWTH WHEN 2-WEEK-OLD PLANTS WERE EXPOSED FOR 1, 2, OR 7 DAYS OR CONTINUOUSLY TO CONCENTRATIONS OF 2,4-DICHLOROPHE-NOXYACETIC ACID IN NUTRIENT SOLUTIONS. MEAN WEIGHTS ARE SHOWN FOR FOUR REPLICATES, EACH CONTAINING TWO PLANTS*

CONCENTRATION (P.P.M.)	PRESENTA-TION TIME (DAYS)	NEW SHOOTS		ROOTS		TOTAL PLANT	
		Final weight (mg.)	Weight increase (%)	Final weight (mg.)	Weight increase (%)	Final weight† (mg.)	Weight increase (%)
0.............	20	560	100	150	100	1190	100
1.............	1	890	160	140	88	1390	125
1.............	2	580	104	150	100	1110	90
1.............	7	290	52	130	75	720	38
1.............	20	80	14	120	63	510	14
5.............	1	230	41	110	50	700	38
5.............	2	510	91	130	75	950	57
5.............	7	60	9	40	....	320	....
5.............	20	20	4	50	....	330	....
20.............	1	110	20	90	25	520	15
20.............	2	210	37	80	13	560	20
20.............	7	30	5	20	....	280	....
20.............	20	10	2	20	....	260	....
Initial dry weight (mg)	....	0	....	70	....	400	....

* Minimum significant difference for mean shoot weight: for experiment at 19:1 odds, 330 mg.; for regulator treat-ments only at 99:1 odds, 100 mg.

† Includes the weight of the old portion of the shoot.

only slight curvature of the stems of some plants occurred. Plants in 20 p.p.m. were wilted by the second day, and there was moderate curvature of stems of some of this group. Such wilted plants recovered turgidity in 1 day if transferred to normal nutrient solution. The leaves, especially the older ones, became chlorotic when plants were exposed for 7

ing of the base of the hypocotyl and of root tips resulted from treatments of 7, 2, and less than 2 days' duration with 1, 5, or 20 p.p.m., respectively. Though some swelling was caused by longer exposure to the higher concentrations, these responses were almost lacking in 20 p.p.m. and were much reduced in 5 p.p.m. The higher-intensity treatments

more nearly arrested all types of development.

The average increase in the total fresh weight of untreated plants was four times the initial weight of the plants. The mean weight of new shoot (weight of shoot above the primary leaf node) comprised a greater fraction of the total increase in weight than that contributed by growth of roots. Seven days of treatment with

dry weight of plants increased less than one-fourth of the normal amount in 20 p.p.m. of 2,4-dichlorophenoxyacetic acid. No increase in plant dry weight occurred when 5 (or more) p.p.m. were presented for 7 (or more) days. All treatments lasting 7 days limited the increase in dry weight of plants to approximately one-half or less of the normal increase. From this test it appears that 5 p.p.m. of 2,4-di-
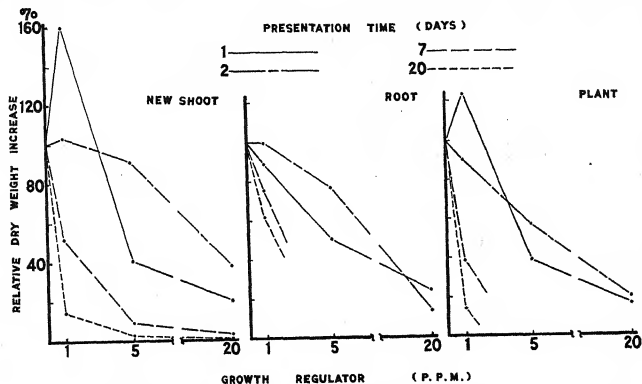
Fig. 1.—The relative increases in the dry weight of soybean plants and plant parts which occurred during the 3d through the 5th weeks of growth when 2-week-old plants were exposed to 2,4-dichlorophenoxyacetic acid in nutrient solution for 1, 2, or 7 days or continuously. The increase in weight of untreated plants, shown as 100 per cent, was 560, 80, and 790 mg. for new shoots, roots, and total plant weights, respectively.

1 or 5 p.p.m., and the 20-p.p.m. treatment for a period as short as 1 day, limited fresh-weight increases of new shoots to less than one-half of normal. Plants which grew in 5 and 20 p.p.m. for 7 days or more were dead at the end of the test period.

Dry-weight data indicate that changes either in concentration or in presentation time resulted in highly significant inhibition of soybean growth in an amount that was, in general, proportional to the treatment intensity (table 1 and fig. 1). The

chlorophenoxyacetic acid presented for slightly more than 2 days will kill young soybeans or completely arrest their growth.

Only the plants treated with 1 p.p.m. for 1 day increased in dry weight more than did untreated plants. In this treatment the decrease in growth of other plant parts was more than equaled by the increase in weight due to the growth of new shoots, which was 1.6 times the normal amount. The weight of hypocotyls and old shoots decreased in high-intens-

ity treatments, while in low-intensity treatments abnormal growth of these parts resulted in subnormal dry-weight increases. The reduction of growth of roots in low-intensity treatments (1 p.p.m.; and 5 or 20 p.p.m. for 2 days

ments were supplied, the symptoms developed by continuously treated plants were similar to those caused by equivalent 2,4-dichlorophenoxyacetic acid concentrations in the observational test on corn. The tips of leaves were slightly

TABLE 2

THE INCREASE IN THE DRY WEIGHT OF CORN PLANTS WHICH OCCURRED DURING THE 3D THROUGH THE 5TH WEEKS OF GROWTH WHEN 2-WEEK-OLD PLANTS WERE EXPOSED FOR 1, 2, OR 7 DAYS OR CONTINUOUSLY TO CONCENTRATIONS OF 2,4-DICHLOROPHE-NOXYACETIC ACID IN NUTRIENT SOLUTIONS. MEANS ARE SHOWN FOR FOUR REPLI-CATES EACH CONTAINING TWO PLANTS*

CONCENTRATION (P.P.M.)	PRESENTA-TION TIME (DAYS)	FINAL WEIGHT AND INCREASE IN WEIGHT OF—					
		Shoots		Roots		Total plant	
		Mg.	%	Mg.	%	Mg.	%
0.............	20	1040	100	330	100	1370	100
1.............	1	980	93	290	85	1370	100
1.............	2	1010	96	370	115	1380	100
1.............	7	530	38	240	67	770	45
1.............	20	390	21	170	41	570	26
5.............	1	680	56	260	74	940	61
5.............	2	560	42	210	55	770	45
5.............	7	320	12	110	19	430	14
5.............	20	280	7	100	15	370	9
20.............	1	660	54	260	74	920	59
20.............	2	290	9	130	26	540	13
20.............	7	170	0	70	4	240	0
20.............	20	250	4	70	4	320	3
Initial dry weight (mg.)	....	220	....	60	....	280	....

* Minimum significant differences for means of total dry weight are as follows:

	99:1 odds	19:1 odds
Presentation time........................	300 mg.	224 mg.
Concentration..........................	260	194
Presentation time × concentration...........	520	387

or less) was, in general, less marked than that caused in shoot growth. However, the character of the root growth which occurred was distinctly abnormal.

In a similar test employing corn plants which had four visible leaves and were approximately 6 inches tall when treat-

wilted on plants exposed for 2 days to 20 p.p.m. and, to a lesser degree, on some plants treated with 5 p.p.m. One day following their transfer to normal nutrient solutions, most plants had recovered turgidity; by 36 hours all signs of wilting had disappeared.

Complete data on fresh weight were not obtainable because shoots of plants exposed for 7 or 20 days to more than 5 p.p.m. of 2,4-dichlorophenoxyacetic acid were partly or completely dead and dry. Roots of these plants were dark colored and watery or soft in appearance. In untreated plants the fresh weight of root, shoot, and whole plant increased in approximately similar proportions, the final

inhibition, which were proportional to treatment intensity (concentration × presentation time), resulted when regulator concentration and presentation time were varied. Shoot growth was 55 per cent or more of normal only in the 1-day treatments and in the 2-day treatment with 1 p.p.m. Treatment with 5 p.p.m. for 7 days or more and with 20 p.p.m. for 2 days or more reduced in-
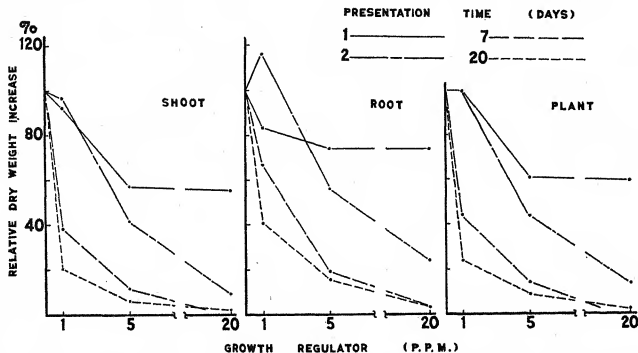
FIG. 2.—The relative increases in the dry weight of corn plants, which occurred during the 3d through the 5th weeks of growth when 2-week-old plants were exposed to 2,4-dichlorophenoxyacetic acid in nutrient solution for 1, 2, or 7 days or continuously. Increases in the dry weight of untreated plants, which are shown as 100 per cent, were 820, 270, and 1090 mg. for shoot, root, and total plant weight, respectively.

weight of which was more than four times that of initial plants. Compared to untreated plants, treatment with 1 p.p.m. for 7 days or 5 p.p.m. for 2 days caused 55–60 per cent decrease in the fresh weight of plants. The fresh weight of shoots increased 30–35 per cent of normal with treatments of 20, 5, or 1 p.p.m. for 1, 2, or 7, days, respectively, but was only 15 per cent of normal when 20 p.p.m. was applied for 2 days.

It is evident from the summary of dry-weight data in table 2 and figure 2 that highly significant differences in growth

creases in the dry weight of shoots by approximately 90 per cent. Again the relative increase in the weight of corn roots (due to proliferation and abnormal development) was greater than that of shoots of treated plants. Increases in dry weight of roots were limited to 25 per cent or less by 5 and 20 p.p.m. presented for 7 and 2 days, respectively.

Abnormal root growth caused more than normal increase in dry weight when plants were treated for 2 days with 1 p.p.m.; however, this increase did not prove to be significant. At the end of the

TABLE 3

THE FRESH AND DRY WEIGHTS OF 5-WEEK-OLD WHEAT PLANTS, TREATED AT THE MIDDLE OF THE THIRD WEEK OF GROWTH FOR 2, 4, 6, OR 17 DAYS WITH 1–4 P.P.M. OF 2,4-DI-CHLOROPHENOXYACETIC ACID. MEAN WEIGHTS ARE SHOWN FOR FOUR REPLICATES, EACH CONTAINING EIGHT PLANTS*

| CONCENTRATION (P.P.M.) | PRESENTATION TIME (DAYS) | FRESH WEIGHT OF: | | | | | |
| | | Shoots | | Roots | | Total plant | |
		Final (gm.)	Increase (%)	Final (gm.)	Increase %	Final (gm.)	Increase (%)
0	17	6.62	100	3.80	100	10.42	100
1	2	5.31	72	4.35	124	9.66	89
1	4	4.96	64	4.17	120	9.13	81
1	6	4.85	62	4.69	129	9.54	87
1	17	2.95	21	2.24	30	5.19	24
2	2	5.54	77	5.14	159	10.68	104
2	4	4.70	58	4.22	118	8.92	78
2	6	3.91	41	4.24	119	8.15	67
2	17	1.97	0	2.03	20	4.00	6
4	2	4.65	57	4.57	134	9.22	83
4	4	4.45	53	4.26	120	8.71	75
4	6	3.80	39	3.88	103	7.68	60
4	17	2.01	0	2.36	35	4.37	12
Initial fresh weight (gm.)	....	2.00	....	1.58	....	3.60	....

| CONCENTRATION (P.P.M.) | PRESENTATION TIME (DAYS) | DRY WEIGHT OF: | | | | | |
| | | Shoots | | Roots | | Total plant | |
		Final (mg.)	Increase (%)	Final (mg.)	Increase (%)	Final (mg.)	Increase (%)
0	17	910	100	190	100	1100	100
1	2	750	77	202	110	952	82
1	4	645	62	205	112	850	70
1	6	660	64	242	142	902	76
1	17	260	7	120	43	380	13
2	2	715	72	232	134	947	82
2	4	580	53	200	108	780	61
2	6	470	37	200	108	670	48
2	17	200	0	92	20	292	2
4	2	600	56	205	112	805	65
4	4	595	55	225	128	820	66
4	6	480	39	212	116	692	63
4	17	210	0	90	19	300	3
Initial dry weight (mg.)	....	210	....	67	....	275	....

* Minimum significant difference for means of shoot dry weight: At 19:1 odds: for the experiment, 125 mg.; for 2,4-dichlorophenoxyacetic acid treatments, 117 mg.

test the appearance of some plants grown continuously in 1 p.p.m. of 2,4-dichlorophenoxyacetic acid and of plants exposed more than 1 day to 5 and 20 p.p.m. strongly suggested that these plants would not continue to grow to maturity if transferred to normal nutrient solution. This test indicated that 2.5–7 days of exposure to concentrations of 2,4-dichlorophenoxyacetic acid of the order of

veloped by plants exposed to higher-intensity treatments differed mainly in that swelling was evident in the crown region. In general, the degree of these responses was proportional to the intensity of the treatment (presentation time × concentration).

At the end of the test period, shoots of plants which had grown in 4 p.p.m. for 6 days or continuously were partly dry.
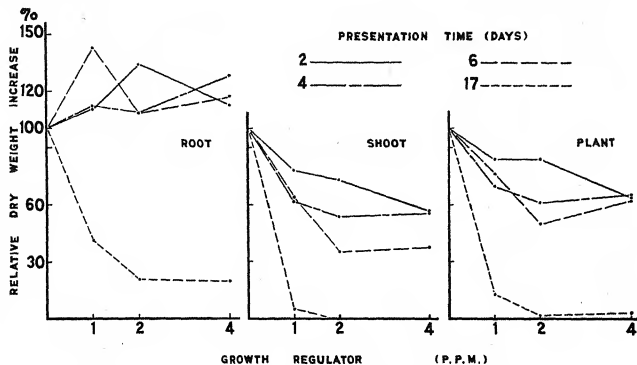
Fig. 3.—The relative increase in the dry weight of wheat plants during the period from the middle of the 3d through the 5th weeks of growth after 17-day-old plants were treated for 2, 4, 6, or 7 days with 1–4 p.p.m. of 2,4-dichlorophenoxyacetic acid in the nutrient solution. The average increases in the weight of root, shoot, and total plant—125, 700, and 825 mg., respectively—are valued as 100 per cent in each case.

5–10 p.p.m. would cause more than 50 per cent reduction in growth of young corn plants.

Two-week-old wheat plants were treated continuously and for 2, 4, or 6 days with 1, 2, or 4 p.p.m. of 2,4-dichlorophenoxyacetic acid in a test which lasted 17 days. On the 12th day of the test period the roots of plants treated with 1 or 2 p.p.m. for more than 1 day were discolored, plainly showed inhibition of elongation and branching, and had localized swellings at the root tips. Symptoms de-

The green and dry weights of untreated plants increased approximately three and four times, respectively, compared to initial plant weights. The data on fresh and dry weights are summarized in table 3, and the relative average increases in dry weight are shown in figure 3. Except for the continuous treatments, which resulted in reductions of from 50 to 75 per cent, the increase in the weight of roots of treated plants was equal to that of control plants. However, roots were distinctly abnormal, swollen, and

soft, and much of the root weight increase was due to stimulated production of adventitious roots.

In proportion to the intensity of the treatments, all treatments caused parallel and significant reductions in dry weight of shoots, which constituted the major part of total plant weight. Continuous treatment with 1–4 p.p.m. resulted in at least 90 per cent reduction in dry weight of plants. When these concentrations were presented for 2 or 4 days, 25–50 per cent of the normal increase in dry weight of plants took place, and these plants appeared capable of further growth. These results suggest that wheat plants, especially with respect to root growth, are relatively tolerant to concentrations of 2,4-dichlorophenoxyacetic acid of the order of 5 p.p.m. presented for periods of time up to 6 days.

Discussion

The relatively greater effectiveness of 2,4-dichlorophenoxyacetic acid on broadleaf plants compared to monocotyledonous species was again borne out by results obtained in these presentation-time studies. Soybean plants were distinctly more inhibited by equivalent treatments with 2,4-dichlorophenoxyacetic acid than were corn or wheat plants. It appeared that in solutions which contained concentrations of the order of 5 p.p.m., soybeans would be killed or their growth completely arrested by exposure for slightly more than 2 days; whereas in similar concentrations appreciably more than 7 days' treatment was necessary to produce similar effects on corn and wheat.

Soybean plants treated for 7 days or longer with 5 p.p.m., soybeans died in less than 2 weeks after treatments were initiated, and no increase in dry weight occurred prior to their death. Though such treatments proved distinctly deleterious to the cereal crops, appreciable increases in the dry weight of plants did result. Treatment for 2 or 7 days with 5 or 1 p.p.m. of 2,4-dichlorophenoxyacetic acid, respectively, resulted in 40–60 per cent reduction of the increase in the dry weight of soybeans, 55 per cent reduction for corn, and, in comparable treatments, from 25 to 35 per cent reduction for wheat.

With respect to growth of roots, wheat plants proved relatively tolerant, and, indeed, increases in weight were induced by treatment with 2,4-dichlorophenoxyacetic acid, but these were due primarily to abnormal development. Such root growth was accompanied by abnormal swelling of the crown region. Both of these responses were again observed to occur to a much lesser extent in corn than in wheat plants (2). However, from the general appearance of plants subjected to comparable treatment, it appeared that wheat plants would be capable of a greater measure of recovery than corn.

In numerous tests conducted in the greenhouse and in the field as a part of other phases of the program on chemical regulation of the growth of plants, 2,4-dichlorophenoxyacetic acid was applied in various ways to the soil, and progressively diminishing amounts of activity were noted to persist in the soil for periods of from 3 to 6 weeks, depending upon the rate of treatment, the character of the soil, and the environmental complex (1). Although the development of plants in soil and in nutrient solutions is not identical, the observations reported in this paper as to effects of root exposure to 2,4-dichlorophenoxyacetic acid for limited periods may be applicable in considering possibilities of injury or recovery in soil in which persistence of the regulator is also limited.

Summary

Observations were made on the development of young vegetative soybean, corn, and wheat plants, which were subjected in nutrient solutions for 1, 2, 6, or 7 days to various concentrations of 2,4-dichlorophenoxyacetic acid. After the desired exposure period had elapsed, such plants were transferred to nutrient solution free of the regulator. Their growth during test periods was compared with that of untreated and continuously treated plants.

Inhibition of the growth of all three crops resulted from treatment for more than 2 days with 1 p.p.m. Abnormal types of growth of shoots and roots of plants occurred in all treatments. The responses induced in roots were more abnormal than were those in shoots.

The growth of cereals was distinctly less inhibited by equivalent treatments than that of soybeans. It appeared that treatment for slightly more than 2 days with 5 p.p.m. would kill or completely arrest the growth of soybeans. Seven days or more of treatment with 5 p.p.m. arrested the growth of the cereals. Wheat was slightly less inhibited than corn by equivalent treatments.

Significant differences in the amount of inhibition of growth resulted from variation of 2,4-dichlorophenoxyacetic acid concentration, length of presentation time, or treatment intensity (concentration $\times$ presentation time). Inhibition was generally proportional to treatment intensity. Exposure for 2 or 7 days to 5 or 1 p.p.m. reduced the increases in the dry weight of soybean plants by 40 and 60 per cent and that of corn by 55 per cent, while comparable treatments resulted in only 25–35 per cent reductions in the weight of wheat plants.

The increase in the weight of shoots of cereals remained nearly normal in low-intensity treatments and was reduced by higher-intensity treatments. More than normal increase in the weight of soybean shoots and plants occurred, following 1 day of treatment with 1 p.p.m.

In all plants 2,4-dichlorophenoxyacetic acid induced an abnormal type of root growth. This effect was at a maximum in low-intensity treatments, such as 1 or 2 days with 5 or 1 p.p.m., and, among the species tested, soybeans were the least affected. Low-intensity treatments significantly increased the dry weight of wheat roots and caused shoots to proliferate and swell at the crown regions. These responses were less marked in corn.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

III. THE RELATIVE TOXICITY OF ISOPROPYLPHENYLCARBAMATE AND SOME PHENOXYACETIC ACID DERIVATIVES TO SOME CEREALS[1]

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Introduction

A series of tests has been conducted with the object of comparing the effects of selected synthetic growth-regulators on the growth of cereals in nutrient-solution cultures. In earlier tests it had been determined that marked quantitative and morphological effects were induced when low concentrations of 2,4-dichlorophenoxyacetic acid were supplied in the nutrient solutions (6, 7). Selected for comparison with 2,4-dichlorophenoxyacetic acid were: ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate (both of which are more soluble in water than the respective acids) and isopropylphenylcarbamate (which is much less water-soluble). In other studies (9), the latter two chemicals showed activity differing from that of 2,4-dichlorophenoxyacetic acid. Isopropylphenylcarbamate, particularly, has high inhibitory activity on cereal seedlings (1).

Some effects of urethanes on the growth of plants have been reported by other investigators. FRIESEN (4) reported distinct effects of low concentrations of ethyl urethane (ethylcarbamate or $C_2H_5OOCNH_2$) on germination and early seedling development of oat and wheat

plants. Ethylphenylurethane in low concentrations has been recently reported to have differential herbicidal effects (8). Cereals were killed or their growth distinctly arrested at all stages of development by a number of aryl carbamic esters and related compounds, which at the same rate of application were without marked effect on dicotyledonous plants. Isopropylphenylcarbamate, which was three times as active as ethylphenylcarbamate, had the greatest activity of the compounds tested.

In recent publications attention has been directed to the selective herbicidal action of 2-methyl-4-chlorophenoxyacetic acid, and its inorganic salts. This substituted acid was stated to be superior to 2,4-dichlorophenoxyacetic acid because of its more precisely selective toxicity to common broadleaf weeds(3, 5).

Methods and materials

Experimental methods used in these tests were in all ways similar to those described in the first report of this series (6).

Experimental results

EXPERIMENT I

Comparison of the effects of 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate on the growth of wheat.

Wheat plants were treated with 2,4-dichlorophenoxyacetic acid during the

4th and 5th weeks of growth. Inhibition of growth of shoots was noticeable after 4 days of treatment with 1.5–3.0 p.p.m. of the acid. On the 6th day the shoots of the plants were chlorotic. Fresh-weight data, which are incomplete for these treatments, since most plants grown in 3 p.p.m. were dead and dry, indicate that, in proportion to the concentration supplied, less than 3 p.p.m. significantly limited the increase in fresh weight of shoots. At 0.05–1 p.p.m. the weight increases were from 80 to 10 per cent of the controls, while in greater concentrations the increases in the fresh weight of shoots were less than 5 per cent of normal.

The fresh weight of roots was significantly greater than normal in 0.10 p.p.m. or less; equal to untreated roots in concentrations up to 0.5 p.p.m.; and decreased to 70 per cent of normal when the concentration was increased to 2 p.p.m. The fresh weight of the total plant was significantly increased by the addition of 0.05 p.p.m. but was progressively decreased as the concentration of 2,4-dichlorophenoxyacetic acid was increased above this amount. Increment increases of 0.25–0.5 p.p.m. were required to result in significant progressive decreases (table 1). The minimum weight was at about 2 p.p.m.

A response of the root system which involved part or all of a series of five types of symptoms was first noticeable on the 3d day. It was distinct by the 6th day of treatment, by which time roots were dark colored in cultures containing more than 0.5 p.p.m. of 2,4-dichlorophenoxyacetic acid. A water-soaked, deteriorated appearance of roots increased in proportion to regulator concentration. The concentration ranges which resulted in development of different symptoms on the root systems of wheat plants were: (1) 0.05 p.p.m., and more: lessened elon-

gation of white fibrous roots until, at 1 p.p.m., no root growth of this type occurred; (2) 0.1 p.p.m., and more: fewer externally visible, normal lateral roots until, at approximately 2 p.p.m., no new branches appeared; (3) 0.1–3 or 4 p.p.m.: development of localized swellings on all root tips, which response was at a maximum at approximately 1.5 p.p.m. and was progressively less in higher concentrations until little swelling occurred in 4 p.p.m. or more; (4) 0.75–4 p.p.m.: stimulated adventitious root growth from the crown regions of plants, which reaction reached a maximum at 2–3 p.p.m. and again was lacking at 4–5 p.p.m.; (5) 1.5–10 p.p.m.: swelling of the crown region, most evident on plants treated with 5 p.p.m. of 2,4-dichlorophenoxyacetic acid. Appreciable crown swelling was caused by 10 p.p.m. before plants were killed.

All isopropylphenylcarbamate concentrations tested limited the increase in fresh weight of wheat treated from the middle of the 3d through the middle of the 5th weeks of growth. The responses of plants and plant parts were similar in the various concentrations, in that fresh weight decreased sharply as concentration was increased from 0.075 to 0.25 p.p.m. Further increase in concentration did not result in further decrease in the fresh weight of plants.

The root systems of plants treated with isopropylphenylcarbamate concentrations greater than 0.075 p.p.m. were discolored. Inhibition of normal elongation and branching was not directly correlated with concentration above 0.25 p.p.m., which was the distinct threshold. In proportion to concentrations of from 0.25 to 5 p.p.m., distinctly less swelling of root tips resulted in comparison with equal concentrations of 2,4-dichlorophenoxyacetic acid. Treatment with iso-

TABLE 1

THE FRESH AND DRY WEIGHTS OF WHEAT PLANTS WHICH WERE GROWN DURING THE 3D THROUGH THE 5TH WEEKS IN NUTRIENT SOLUTIONS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID OR ISOPROPYLPHENYLCARBAMATE. WEIGHTS ARE MEANS FOR EIGHT PLANTS IN EACH OF QUADRUPLICATE CULTURES TREATED WITH 2,4-DICHLOROPHENOXYACETIC ACID (A) AND OF TRIPLICATE CULTURES TREATED WITH ISOPROPYLPHENYLCARBAMATE (B). CURVES 1 AND 2 IN FIGURE 1 SHOW THE DRY-WEIGHT DATA PRESENTED IN THIS TABLE UNDER A AND B, RESPECTIVELY.

REGULATOR (P.P.M.)	FINAL FRESH WEIGHT (GM.)*						PER CENT INCREASE IN FRESH WEIGHT					
	Shoots		Roots		Total		Shoots		Roots		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	6.31	24.77	2.69	14.80	9.00	39.57	100	100	100	100	100	100
0.05	5.55	......	3.74	......	9.29	......	81	.....	184	.....	106	.....
0.075	......	21.36	......	13.74	......	35.10	.....	85	.....	91	.....	87
0.10	4.11	......	3.28	......	7.38	......	45	.....	145	.....	69	.....
0.25	3.31	6.69	2.72	4.07	6.03	10.76	25	17	102	6	44	13
0.50	3.41	7.36	2.74	4.41	6.15	11.77	28	20	104	9	46	16
0.75	2.87	5.83	2.52	3.96	5.39	9.79	14	12	87	5	32	10
1.00	2.70	5.64	2.61	3.90	5.31	9.53	10	12	94	4	30	10
1.50	2.25	6.86	2.38	4.29	4.63	11.15	0	18	75	8	17	14
2.00	2.42	......	2.33	......	4.74	......	3	.....	.....	.....	19	.....
2.50	......	6.42	......	4.69	......	11.11	.....	16	.....	11	.....	14
5.00	Dead	5.33	Dead	4.28	Dead	9.61	Dead	11	Dead	7	Dead	10
Initial fresh weight (gm.)	2.32	3.01	1.41	3.44	3.73	6.45	.....	.....	.....	.....	.....	.....

REGULATOR (P.P.M.)	FINAL DRY WEIGHT (MG.)*						PER CENT INCREASE IN DRY WEIGHT					
	Shoots		Roots		Total		Shoots		Roots		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
0.00	690	3187	113	753	803	3940	100	100	100	100	100	100
0.05	640	......	138	......	778	......	89	.....	142	.....	95	.....
0.075	......	3490	......	763	......	4253	.....	111	.....	102	.....	110
0.10	520	......	128	......	648	......	63	.....	125	.....	70	.....
0.25	393	1497	143	379	535	1890	35	43	150	38	49	39
0.50	415	1580	143	427	558	2007	40	42	150	43	53	42
0.75	358	1290	136	353	493	1643	27	31	138	30	40	31
1.00	353	1170	150	360	503	1530	26	27	164	42	42	28
1.50	288	1447	125	390	413	1843	12	37	121	37	25	37
2.00	308	......	125	......	433	......	17	.....	123	.....	29	.....
2.50	......	1397	......	390	......	1787	.....	35	.....	37	.....	36
3.00	248	......	113	......	360	......	4	.....	100	.....	15	.....
4.00	255	......	105	......	360	......	5	.....	88	.....	15	.....
5.00	238	983	105	340	343	1323	1	20	88	28	11	22
10.00	245	......	75	......	320	......	3	.....	38	.....	7	.....
Initial dry weight (mg.)	233	430	53	180	286	610	.....	.....	.....	.....	.....	.....

*Minimum significant difference in mean weight at 10:1 odds: Fresh weight (gm.): shoots (A) 0.88; roots (A) 0.58; total plant (A) 0.13, (B) 4.15. Dry weight (mg.): shoots (A) 131; roots (A) 40; total plant (A) 121, (B) 610.

propylphenylcarbamate resulted in no stimulation of adventitious root growth and relatively little swelling of the crown region of plants.

The response of wheat plants to isopropylphenylcarbamate differed sharply from that caused by 2,4-dichlorophenoxyacetic acid, in that all plants treated with the former remained alive throughout the test period. Growth of shoot was

in 2,4-dichlorophenoxyacetic acid solution, in no case was there a significant increase in total weight or weight of plant parts. More than 0.10 p.p.m. of either compound significantly decreased the dry weight of all plant parts. Isopropylphenylcarbamate limited growth slightly more than 2,4-dichlorophenoxyacetic acid in the concentration range 0.10–1.0 p.p.m. (fig. 1); but when the concentra-
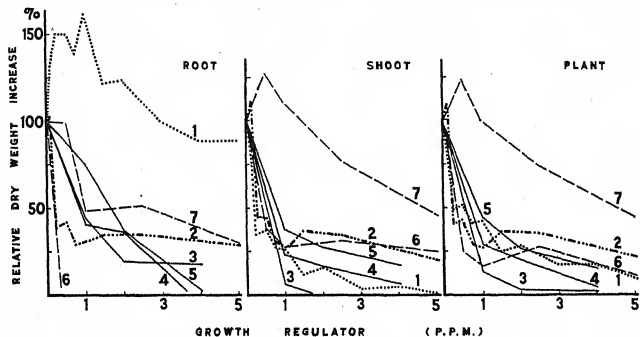
FIG. 1.—The relative increases in the dry weights of wheat and rice plants grown continuously in nutrient solutions containing growth-regulators: Curves are labeled: (for expt. I on wheat) *1*, 2,4-dichlorophenoxyacetic acid; *2*, isopropylphenylcarbamate; (for expt. II on wheat) *3*, 2,4-dichlorophenoxyacetic acid; *4*, ammonium 2-methyl-4-chlorophenoxyacetate; *5*, ammonium 2,4-dichlorophenoxyacetate; (for expt. III on rice) *6*, 2,4-dichlorophenoxyacetic acid; and *7*, isopropylphenylcarbamate. The dry-weight increases in the absence of growth-regulator are valued as 100 per cent.

distinctly inhibited by more than 0.075 p.p.m. However, the degree of inhibition was not distinctly proportional to concentration above 0.25 p.p.m. Although this substance inhibited growth of the main shoot, it also appeared to stimulate the production of small tillers. The maximum number of these occurred at less than 0.25 p.p.m. Shoots of plants were distinctly dark green, appeared highly cutinized, and were stiff or leathery to the touch.

Complete dry-weight data (table 1) showed that, except for growth of roots

tion range was increased above 2 p.p.m., the added increments of the latter depressed growth relatively more than similar added increments of the former. Very low concentrations (0.075 p.p.m.) of isopropylphenylcarbamate stimulated tiller growth. Abnormal growth of wheat-roots was stimulated by 2,4-dichlorophenoxyacetic acid concentrations up to 2 p.p.m.

EXPERIMENT II

Comparison of the effects of 2,4-dichlorophenoxyacetic acid, ammonium 2,4-dichlorophenoxyacetate, and ammo-

nium 2-methyl-4-chlorophenoxyacetate on the growth of wheat.

Several lots of wheat plants, 2 weeks old, growing in nutrient solution, were treated with concentrations of 1, 2, or 4 p.p.m. of 2,4-dichlorophenoxyacetic acid, ammonium 2,4-dichlorophenoxyacetate, or ammonium 2-methyl-4-chlorophenoxyacetate. All plants receiving 1 or 4 p.p.m. and half of the plants receiving 2 p.p.m. of the respective growth-regulators were exposed continuously for 17 days to these concentrations. The other half of the plants supplied with solutions containing 2 p.p.m. were grown for only 4 days in these solutions and were then removed to normal nutrient solution and grown there for 13 days more.

At the end of the 17-day test period all plants were alive. Parts of shoots were dry in all cultures containing 4 p.p.m. of any one of the growth-regulators. In those treated continuously with 2 p.p.m. of 2,4-dichlorophenoxyacetic acid or ammonium 2-methyl-4-chlorophenoxyacetate, parts of shoots were similarly dry. Roots in all cultures on continuous treatment containing 2 p.p.m. or more of any of the growth-regulators were in poor condition. It appeared likely that a few of the plants treated with 4 p.p.m., and some treated continuously with 2 p.p.m., would be able to grow to maturity.

In proportion to concentration, all three growth-regulators caused darkening of the root systems and swelling of root tips and the crown regions of plants and stimulated the production of adventitious roots. 2,4-Dichlorophenoxyacetic acid, ammonium 2-methyl-4-chlorophenoxyacetate, and ammonium 2,4-dichlorophenoxyacetate ranked in decreasing order in these respects.

Among the cultures treated with 1 p.p.m., only 2,4-dichlorophenoxyacetic acid caused crown swelling. Roots of plants grown in ammonium 2,4-dichlorophenoxyacetate were relatively branched and elongated. The roots of plants grown in ammonium 2-methyl-4-chlorophenoxyacetate were the least discolored of those in 2-p.p.m. treatments, while ammonium 2,4-dichlorophenoxyacetate caused the greatest stimulation of adventitious root growth. Little stimulation of adventitious roots was evident in any culture treated with 4 p.p.m. The much-inhibited root systems of plants treated with 2-methyl-4-chlorophenoxyacetate showed slightly less deterioration than those of plants exposed to 4 p.p.m. of the other regulators.

Shoots of plants exposed for only 4 days to 2 p.p.m. of the growth-regulators were green at the end of the test period. In all cases the root systems were slightly discolored, and slight swelling of the root tips was evident. Only the 4-day treatment with 2,4-dichlorophenoxyacetic acid resulted in marked swelling of crowns. Crowns of plants treated with 2-methyl-4-chlorophenoxyacetate were slightly swollen. Similar treatments with ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate stimulated the branching and elongation of roots compared to untreated and 2,4-dichlorophenoxyacetic acid-treated plants. The most stimulation resulted from ammonium 2,4-dichlorophenoxyacetate. At the end of the test it appeared that plants treated for 4 days with 2 p.p.m. of these growth-regulators would be able to continue growth to maturity.

Since parts of some treated plants were dry and since changes in the green weights of plants subjected to these growth-regulators were quite similar to the increases in dry weights, only dry-weight data are presented for this test

(table 2). The weight of shoots and plants was significantly decreased by all regulators in proportion to the concentration used, except in the 4-day treatments with ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate. Root growth was stimulated with all three of the growth-

Increase in the dry weight of shoots was inhibited slightly more than increase in weight of roots by these chemical regulators (fig. 1). In general, 2,4-dichlorophenoxyacetic acid concentrations caused greater inhibition of growth than did equal treatments with either of the ammonium salts. The amount of growth

TABLE 2

THE DRY WEIGHT OF WHEAT PLANTS GROWN DURING THE 3D THROUGH THE 5TH WEEKS IN NUTRIENT SOLUTIONS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID (3), AMMONIUM 2-METHYL-4-CHLOROPHENOXYACETATE (4), OR AMMONIUM 2,4-DICHLOROPHENOXYACETATE (5). MEAN WEIGHTS ARE FOR FOUR REPLICATES, EACH CONTAINING EIGHT PLANTS

REGULATOR TESTED	CONCENTRATION (P.P.M.)	PRESENTATION TIME (DAYS)	SHOOTS		ROOTS		TOTAL	
			Final weight (mg.)*	Increase (%)	Final weight (mg.)*	Increase (%)	Final weight (mg.)*	Increase (%)
Control.........	0	....	910	100	190	100	1100	100
5..............	2	4	800	84	295	185	1095	99
4..............	2	4	690	69	255	153	945	81
3..............	2	4	580	53	200	108	780	61
5..............	1	17	465	36	160	75	625	42
	2	17	370	23	110	35	480	25
	4	17	330	17	70	3	400	15
4..............	1	17	385	25	115	39	500	27
	2	17	325	16	110	35	435	19
	4	17	250	6	60	0	310	5
3..............	1	17	260	7	120	43	380	13
	2	17	200	0	95	20	295	2
	4	17	210	0	90	19	300	3
Initial weight (mg.)........			210	....	67	....	275	....

* The minimum significant difference in mean shoot weight for treated plants is 85 mg. The minimum significant difference in mean total plant weight for 4-day treatments with 2 p.p.m. (only) is 255 mg.

regulators in the 4-day treatments. The greatest stimulation was caused by ammonium 2,4-dichlorophenoxyacetate. Among the 4-day treatments the least stimulation and most abnormal type of root growth was caused by 2,4-dichlorophenoxyacetic acid. All the continuous treatments decreased the weight of roots in proportion to concentration of regulator.

which occurred in the ammonium 2,4-dichlorophenoxyacetate treatments was notably greater than that in equivalent concentrations of 2,4-dichlorophenoxyacetic acid or ammonium 2-methyl-4-chlorophenoxyacetate.

EXPERIMENT III

Comparison of the effects of 2,4-dichlorophenoxyacetic acid and isopropyl-

phenylcarbamate singly or in mixtures on the growth of rice plants.

Rice plants which had been grown in sand for 9 weeks were mounted in solution cultures containing 0.00022 M. calcium nitrate, 0.00012 M. monopotassium acid phosphate, 0.00012 M. magnesium sulphate, 0.00004 M. ammonium sulphate; and the following p.p.m. concentrations of minor elements: 0.5 of iron, 0.25 of boron, 0.25 of manganese, 0.01 of cobalt, and 0.025 of zinc. When the plants were 10 weeks old, the cultures were supplied with this nutrient solution with the addition of 0.5, 1.0, 2.5, or 5.0 p.p.m. of 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate. Cultures were also treated with 1-, 2.5-, and
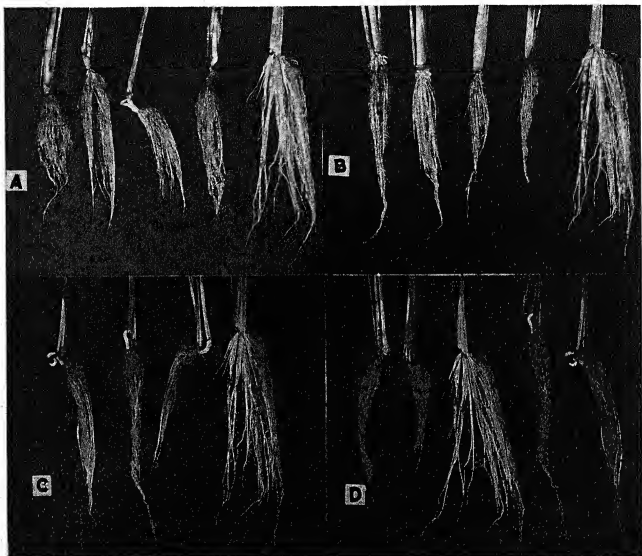
Fig. 2.—Root systems and the crown regions of rice plants, 14 weeks old, grown continuously after the 10th week in nutrient solutions containing growth-regulators. From left to right the treatments supplied to plants shown were A, 0.5, 1.0, 2.5, 5.0 p.p.m. of 2,4-dichlorophenoxyacetic acid, and control. (Note: on treated plants, dark color, inhibition of elongation and branching of roots, swollen crowns; and swollen root tips in 0.5 p.p.m.) B, 0.5, 1.0, 2.5, 5.0 p.p.m. of isopropylphenylcarbamate, and control. (Note: stimulated production of adventitious roots in 0.5–2.5 p.p.m., slightly swollen crowns, and the slightly darker color of treated roots.) C, 1.0-, 2.5-, and 5.0-p.p.m. mixtures of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in 1:3 proportion, and control. (Note: dark color, and decreased elongation and branching of roots; few adventitious roots, and swollen crowns on treated plants.) D, 1.0- and 2.5-p.p.m. mixtures of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in 1:1 proportion, and control, and 2.5- and 1.0-p.p.m. mixtures of these agents, respectively, in 1:3 proportion. (Note: less swelling of the crown region and more growth of adventitious roots on the treated plant at the right.)

5-p.p.m. mixtures of these growth-regulators in the proportions 1:3, 1:1, and 3:1.

These older rice plants developed symptoms which, in general, were similar to those described for wheat (expts. I and II), in response to equivalent concentrations of 2,4-dichlorophenoxyacetic acid in the nutrient solution. All plants were alive at the end of the 4-week test period. Parts of shoots were dry, and the swelling of crowns was much decreased in cultures which contained 2.5 p.p.m. or more of this regulator.

All treatments caused discoloration of roots and inhibited their elongation. Maximum typical swelling of root tips and crowns was caused by treatments with 0.5–1.0 p.p.m. and by 1.0–2.5 p.p.m. of 2,4-dichlorophenoxyacetate acid, respectively. No swelling of root tips and little or no swelling of the crowns of plants occurred in solutions containing isopropylphenylcarbamate. Plants treated with 0.5 and 1.0 p.p.m. of this regulator developed abnormal numbers of adventitious roots.

The general appearance of plants treated with mixtures of these regulators indicated that the effect of 2,4-dichlorophenoxyacetic acid predominated over that of isopropylphenylcarbamate. Stimulation of tillering and the development of stunted, dark-green, leathery leaves, similar to the responses noted when wheat was treated with isopropylphenylcarbamate, were not evident in rice plants. Some of the changes which were induced by these treatments are evident on the representative 14-week-old plants which appear in figures 2 and 3.

The data on dry weight (table 3) indicate that 2,4-dichlorophenoxyacetic acid treatment resulted in distinctly greater inhibition of the vegetative growth of rice than equivalent concentrations of isopropylphenylcarbamate. All treatments, except 0.5 and 1.0 p.p.m. of isopropylphenylcarbamate, caused significant inhibition of the growth of rice plants or plant parts (fig. 1). Shoot growth was significantly increased by 0.5 p.p.m. of isopropylphenylcarbamate, which did not alter the weight of roots from normal. One p.p.m. of isopropyl-

FIG. 3.—Crown regions and portions of the root systems of rice plants treated with 1 p.p.m. of 2,4-dichlorophenoxyacetic (left) or isopropylphenylcarbamate (right). (Note: differences in color and general vigor of the root systems, swollen deteriorated crown of the plant on the left, and greater growth of adventitious roots from the crown region of the plant on the right.)

phenylcarbamate decreased the weight of roots but did not affect the increase in weight of shoots.

Treatment with any of the various mixtures of growth-regulators did not result in more pronounced effects on the growth of plants than those caused by equivalent concentrations of 2,4-dichlorophenoxyacetic acid alone. These data indicate possible, but only slight, supplemental inhibitory effects of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid supplied in mixtures. The amount of inhibition of rice growth

in any mixture most nearly corresponded to that which resulted in 2,4-dichlorophenoxyacetic acid equivalent to the concentration of that component in the mixture.

Discussion

In concentrations as low as 0.25–1.0 p.p.m., all substances tested were highly active in affecting the growth of cereals in nutrient-solution culture. Low con-

centrations, which resulted in general growth inhibition, also stimulated marked changes in the character of the root systems. Swelling of root tips of cereals in these tests closely resembled the morphological response of the roots of sweet peas, the shoots of which were treated with 4-chlorophenoxyacetic acid (2).

The ability of cereals to accomplish a relatively great amount of such abnormal root growth may, in part, account for the

TABLE 3

THE AVERAGE DRY WEIGHTS OF RICE PLANTS GROWN IN NUTRIENTS CONTAINING 2,4-DICHLOROPHE-
NOXYACETIC ACID (6), ISOPROPYLPHENYLCARBAMATE (7), OR MIXTURES OF THESE REGULATORS.
MEAN WEIGHTS ARE OF QUADRUPLICATE CULTURES, EACH CONTAINING FOUR PLANTS

GROWTH-REGULATOR TREATMENT				SHOOTS		ROOTS		TOTAL	
(7) (p.p.m.)	(6) (p.p.m.)	Total (p.p.m.)	Proportion (7):(6)	Final weight (mg.)	Increase (%)	Final weight (mg.)	Increase (%)	Final weight (mg.)	Increase (%)
Control	....	0.0	....	5040	100	1578	100	6620	100
....	0.5	0.5	....	2832	38	753	0	3585	25
....	1.0	1.0	....	2388	26	778	0	3165	16
....	2.5	2.5	....	2645	32	993	3	3638	28
....	5.0	5.0	....	2385	25	1043	12	3428	23
0.5	....	0.5	....	6023	127	1575	99	7598	124
1.0	....	1.0	....	5360	109	1258	48	6618	99
2.5	....	2.5	....	4260	78	1283	52	5543	74
5.0	....	5.0	....	3115	45	1138	28	4253	43
0.25	0.75	1.0	1:3	2385	24	938	0	3323	20
0.50	0.50	1.0	1:1	2488	27	848	0	3335	20
0.75	0.25	1.0	3:1	2555	29	1023	9	3578	26
0.63	1.87	2.5	1:3	2225	20	828	0	3053	13
1.25	1.25	2.5	1:1	2008	14	845	0	2853	9
1.87	0.63	2.5	3:1	2245	21	973	1	3218	18
1.25	3.75	5.0	1:3	2048	15	968	0	3015	13
2.50	2.50	5.0	1:1	2405	25	983	2	3388	22
Initial weight (mg.)..	....	....	....	1532	....	969	....	2501	....

	Shoots	Roots	Total
Minimum significant difference of mean weight (mg.) at odds of:			
99:1....................	930	505	1290
19:1....................	690	380	965

differential action of soil treatments with substituted phenoxyacetic acids. Maintained during such treatment periods by abnormal growth of the root systems, stimulated production of adventitious roots, and abnormal crown development, cereals could later, to greater extent, resume and continue more normal growth.

These tests did not clearly indicate any marked difference in the toxicity of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid to growth of young cereal plants. Increase in the amount of growth of shoots occurred in very low concentrations of the former, while with from 0.25 to 2.0 p.p.m. the growth of wheat was inhibited slightly more than with 2,4-dichlorophenoxyacetic acid. Two p.p.m. or more limited the growth of wheat slightly less than equivalent concentrations of 2,4-dichlorophenoxyacetic acid. The latter inhibited the growth of rice more than isopropylphenylcarbamate in all concentrations tested.

The appearance of plants which were treated with these growth-regulators was distinctly different, in that 2,4-dichlorophenoxyacetic acid treatments resulted in parts of shoots withering or dying; whereas, throughout the test periods, the shoots arrested by isopropylphenylcarbamate remained alive, becoming dark-green and highly cutinized or leathery. Such response to isopropylphenylcarbamate has been previously reported by others (8), who also describe a crown-swelling response, which occurred following treatment of cereals with this substance. Little swelling of crowns resulted from treatments with this regulator in nutrient solution, but low concentrations stimulated the production of small tillers on wheat and of adventitious roots on older rice plants.

Recently it has been suggested that 2-methyl-4-chlorophenoxyacetic acid was more precisely selective when used as a herbicide to control growth of weeds growing with cereals (3). In these nutrient-solution tests the ammonium salt of this growth-regulator appeared slightly, and in some instances significantly, less toxic to wheat than equivalent treatments with 2,4-dichlorophenoxyacetic acid. Of the three phenoxyacetic acid derivatives studied, ammonium 2,4-dichlorophenoxyacetate was the least toxic to cereals.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

IV. THE AMOUNT OF GROWTH IN SOIL AND SOLUTION CULTURES TREATED WITH EQUAL WEIGHTS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE[1]

D. L. TAYLOR, MAJ., A.U.S.

Introduction

In the course of the work described in the preceding papers (3, 4, 5), it was observed that phenoxyacetic acids presented in nutrient-solution cultures caused greater inhibition to the growth of plants than was caused by equal or larger amounts of such growth-regulators applied as soil treatments. Studies were therefore made to determine the magnitude of the influence of soil on the action of ammonium 2,4-dichlorophenoxyacetate by supplying equal weights of this growth-regulator in soil and in solution cultures.

Methods and materials

The soil cultures consisted of 1-gallon glazed crocks, each of which contained an air dry weight of 3770 gm. of thoroughly mixed sand, compost, and field topsoil in the proportions of 1:1:2. The soil moisture level in these cultures was maintained at 32.7 per cent (on the basis of the air dry weight of the soil mixture, or 1235 gm./culture) by weighing the cultures twice daily and adding water as required. An equal volume was maintained in nutrient-solution cultures by

adding twice daily the required amounts of distilled water.

The solution-culture crocks were filled with fresh nutrient solution just prior to the time when the growth-regulator was added. Thereafter no more nutrient was added to the cultures, which were aerated twice daily during the test period. The nutrient solution and, in all other respects, the management of these cultures were the same as those described in the first paper of this series (3).

When the plants were 2 weeks old and established in the soil and solution cultures, aliquots of growth-regulator stock solution containing the desired weight of regulator were evenly applied to the surface of the soil or thoroughly mixed with the solution cultures. The regulator was placed in the soil cultures just prior to the addition of water, in order that the water might carry the regulator solution down through the soil. Quadruplicate cultures of each type, which contained three red kidney beans or soybeans or eight oat plants, were treated with 2.5, 5, and 10 mg. of ammonium 2,4-dichlorophenoxyacetate. These amounts were calculated to give theoretical initial concentrations of approximately 2, 4, or 8 p.p.m. The plants were allowed to grow for a period of 8 days after treatment, at which time data on dry weight were obtained.

Results

During the test period, responses of root systems similar to those previously described for treatments with ammonium 2,4-dichlorophenoxyacetate (5) were observed on all three species in nutrient solutions containing the regulator. Swelling of the hypocotyl region and stimulated growth of adventitious roots occurred on all kidney beans treated in solution cultures and on plants in soil cultures which were treated with 10 mg. The root systems of all plants were much stunted in all solution-culture treatments. All treatments of soybeans and oats resulted in only slight swelling of the hypocotyl and crown region.

Applications of 8 p.p.m. to solution cultures resulted in death of kidney beans and soybeans by the 6th day, while oat plants similarly treated were in poor condition. All plants were dead or partly dry in solution cultures containing 4 p.p.m., and even at 2 p.p.m. in solution culture some of the kidney beans and soybeans were dead. The plants in soil cultures presented a sharp contrast in appearance. Only when 10 mg. of ammonium 2,4-dichlorophenoxyacetate was added was there swelling or drying of parts of the plants. Most of the kidney beans and soybeans in the other treatments and all oat plants survived and at the end of the experiment appeared to be capable of continued growth.

The dry-weight data (table 1) indicate that the growth of these crops in soil or solution cultures was inhibited by all treatments in proportion to the concentration of the growth-regulator applied. In comparison to the effects in solution cultures, the effects of the several treatments in soil cultures were distinctly less acute. In general, the relative growth of plants in the light soil used was approximately four to five times that which oc-

TABLE 1

THE EFFECT OF EQUAL AMOUNTS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE APPLIED IN SOIL AND IN SOLUTION CULTURES ON THE GROWTH OF OATS, KIDNEY BEANS, AND SOYBEANS. VALUES ARE MEANS OF DRY WEIGHT OF PLANTS (EIGHT OAT, THREE KIDNEY BEAN, OR THREE SOYBEAN PLANTS) IN QUADRUPLICATE CULTURES*

Regulator treatment		Soil		Solution	
		Final	Increase†	Final	Increase†
Mg.	P.p.m.	Gm.	%	Gm.	%
Oat cultures					
0.0	0.0	2.30	100	1.59	100
2.5	2.0	2.22	96	0.73	19
5.0	4.0	1.98	83	0.69	15
10.0	8.0	1.62	64	0.75	21
Initial weight (gm.)		0.42		0.53	
Kidney bean cultures					
0.0	0.0	3.07	100	0.98	100
2.5	2.0	2.09	64	0.43	5
5.0	4.0	1.18	30	0.34	0
10.0	8.0	0.57	8	0.28	0
Initial weight (gm.)*		0.36		0.40	
Soybean cultures					
0.0	0.0	1.34	100	0.30	100
2.5	2.0	0.88	61	0.17	35
5.0	4.0	0.83	57	0.13	15
10.0	8.0	0.53	31	0.11	6
Initial weight (gm.)*		0.17		0.10	

* Values shown for kidney beans and soybeans represent the average weight of the trifoliate leaves only.

† For evaluation of the relative increases in weight, that of untreated plants in each type of culture is valued at 100 per cent.

curred in solution cultures containing equal amounts, or theoretical concentrations, of ammonium 2,4-dichlorophenoxyacetate. Conversely, the relative amount of growth of plants in treated cultures was approximately the same when the amount of growth-regulator applied to soil cultures was four times that applied in solution cultures. In these tests, run concurrently, oats were the least affected and kidney beans the most inhibited by the treatments applied either to soil or in nutrient cultures.

The growth of wheat, clover, and sugar-beet plants in that order was recently reported to be increasingly affected by soil treatment with 2,4-dichlorophenoxyacetic acid. In a light, sandy soil which was low in organic matter, this growth-regulator markedly inhibited the development of these crops. The growth of clover, and especially that of wheat, was much less arrested when equivalent treatments were given in soil high in organic matter (2). Similar effects of organic matter on the toxicity of this regulator to kidney beans and other crops were observed in the general program of these investigations (1). It seems probable that some such relationship obtained in the light-soil mixture employed in the comparative tests described herein and that, in a soil of higher organic and clay content, the differences in the intensity of the responses produced in soil and nutrient culture might be even greater. No explanation is yet available for the apparent retention or inactivation of the growth-regulator by the organic or colloid components of the soil. The observations reported above raise the possibility of controlling water plants by applications that might not affect other plants growing in soil along the margins.

Summary

Red kidney beans, soybeans, and oat plants, 2 weeks old, were grown in soil and solution cultures which contained equal volumes of water and were treated with equal weights of ammonium 2,4-dichlorophenoxyacetate.

Morphological changes in plants were induced and growth of all species was arrested by all treatments in proportion to the concentration of the regulator supplied.

The effects of equivalent treatments on these crops increased in the following order: oats, soybeans, and kidney beans. Oats were distinctly less affected than the broadleaved species.

When equivalent amounts of ammonium 2,4-dichlorophenoxyacetate were supplied, approximately four to five times as much inhibition of growth occurred in solution cultures as in cultures containing the light-soil mixture used in these tests.

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